Supplementary Information

Enantiodivergent α-Amino C–H Fluoroalkylation Catalyzed by Engineered Cytochrome P450s

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I. General Methods

1.1 General. Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers (Sigma-Aldrich, VWR, Alfa Aesar, Acros, etc.) and used without further purification. Silica gel chromatography was carried out using AMD Silica Gel 60, 230-400 mesh. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Prodigy 400 MHz instrument (400 MHz for ^{1}H and 101 MHz for ^{13}C NMR) or a Varian 300 MHz Spectrometer (300 MHz for ^{1}H NMR). Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane, using the solvent resonance as the internal standard (^{1}H NMR: δ = 7.26, ^{13}C NMR: δ = 77.36 for $CDCl_3$). ^{19}F NMR data were collected on a Varian 300 MHz spectrometer (282 MHz for ^{19}F NMR). Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets), coupling constant (Hz), integration. Sonication was performed using a Qsonica Q500 sonicator. High-resolution mass spectra were obtained at the California Institute of Technology Mass Spectral Facility.

E. coli cells were grown using Luria-Bertani medium or HyperBroth (AthenaES) with 100 μg/mL ampicillin (LB_{amp} or HB_{amp}). Primer sequences are available upon request. T5 exonuclease, Phusion polymerase, and *Taq* ligase were purchased from New England Biolabs (NEB, Ipswich, MA). M9-N minimal medium (abbreviated as M9-N buffer; pH 7.4) was used as a buffering system for whole cells, lysates, and purified proteins, unless otherwise specified. M9-N buffer was used without a carbon source; it contains 47.7 mM Na_2HPO_4, 22.0 mM KH_2PO_4, 8.6 mM NaCl, 2.0 mM $MgSO_4$, and 0.1 mM $CaCl_2$.

1.2 Chromatography. Gas chromatography-mass spectrometry (GC-MS) analyses were carried out using Shimadzu GCMS-QP2010SE system and J&W HP-5ms column. Analytical chiral HPLC was conducted using either an Agilent 1200 series instrument with *n*-hexane and isopropanol as the mobile phase. Enantiomers were separated using one of the following chiral columns: Chiralpak IA (4.6 mm × 25 cm), Chiralpak IC (4.6 mm × 25 cm), Chiralpak AD (4.6 mm × 25 cm), Chiralcel OJ-H (4.6 mm × 25 cm). Chiral GC was performed on an Agilent 6850 GC with FID detector using a Astec-CHIRALDEX G-TA column (30.0 m × 0.25 mm) at 1.0 mL/min He carrier gas flow.

1.3 Cloning and site-saturation mutagenesis. The genes encoding all enzymes described in this study were cloned using Gibson assembly[1] into vector pET22b(+) (Novagen) between restriction sites *Nde*I and *Xho*I in frame with a *C*-terminal 6xHis-tag. Site-saturation mutagenesis was performed using the "22c-trick" method.[2] The PCR products were digested with *Dpn*I, gel purified, and ligated using Gibson Mix™.[1] The ligation mixture was used to directly transform electrocompetent *E. coli* strain *E. cloni* BL21 (DE3) cells (Lucigen).

1.4 Expression of P450 and P411 variants in 96-well plates. Single colonies from LB_{amp} agar plates were picked using sterile toothpicks and cultured in deep-well 96-well plates containing LB_{amp} (400 μL/well) at 37 °C , 80% humidity and 250 rpm shaking overnight. Subsequently, HB_{amp} (1080 μL/well) in a deep-well plate was inoculated with an aliquot (120 μL/well) of these overnight cultures and allowed to shake for 3 hours at 37°C , 80% humidity and 250 rpm. The plates were then cooled on ice for 30 minutes and the cultures were induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 1.0 mM 5-aminolevulinic acid (ALA) (final concentrations). Expression were then conducted at 20 °C, 230 rpm for 18-20 hours.

1.5 Plate reaction screening in whole-cell format. *E. coli* cells harboring P411 variants in

deep-well 96-well plates were pelleted (3,500 × g, 5 min, 4 °C) and resuspended in M9-N buffer (375 μL) by gentle vortexing. The 96-well plates were then transferred to an anaerobic chamber. To deep-well plates of cell suspensions were added the *N*-phenylpyrrolidine substrate **1a** (10 μL/well, 400 mM in EtOH) and 2,2,2-trifluoro-1-diazoethane **2** (15 μL per well, 600 mM in EtOH). During the addition, the stock solution of 2,2,2-trifluoro-1-diazoethane **2** is kept on an ice-salt bath (-20 °C) to minimize evaporation. The plates were sealed with aluminum sealing foil immediately after the addition and shaken in the anaerobic chamber at room temperature and 700 rpm. After 18-24 hours, the seals were removed and 610 μL 1:1 ethylacetate/hexanes solution containing 0.66 mM 1,3,5-trimethoxybenzene internal standard was added. The plates were tightly sealed with silicone mats, vigorously vortexed, and centrifuged (5,000 × g, 5 min) to completely separate the organic and aqueous layers. The organic layer of each well was then transferred to individual vials equipped with autosampler vial inserts and analyzed by GC-MS. To prepare samples for enantioselectivity determination via normal-phase chiral HPLC, the organic solution in the vial was removed under reduced pressure and the resulting crude product was re-dissolved in hexanes.

1.6 Expression of P411 variants and cell lysate preparation. *E. coli* (*E. cloni* BL21(DE3)) cells carrying plasmid encoding the appropriate P411 variant were grown overnight in 5 mL LB_{amp}. Preculture (3 mL) was used to inoculate 27 mL of HB_{amp} in a 125 mL Erlenmeyer flask; this culture was incubated at 37 °C, 230 rpm for 2.5 hours. The culture was then cooled on ice for 30 min and induced with 0.5 mM IPTG and 1.0 mM ALA (final concentrations). Expression was conducted at 20 °C, 150 rpm for 16-18 hours. Subsequently, the *E. coli* cells were pelleted by centrifugation (3,000 × g, 5 min, 4 °C). Media was removed and the resulting cell pellet was resuspended in M9-N buffer to OD_{600} = 30. The cell suspension was then lysed by sonication using a Qsonica Q500 sonicator equipped with a microtip (2 minutes, 1 second on, 1 second off, 30% amplitude); samples were kept on wet ice for this process. The resulting lysed solution was centrifuged (20,000 × g, 10 min, 4 °C) to remove cell debris. The supernatant (clarified lysate) was separated from the pellet and kept on ice until use.

1.7 Hemochrome assay for the determination of heme protein concentration. The concentration of heme protein in the clarified lysate was determined by the hemochrome assay.[3] Briefly, 500 μL of the lysate was added to a cuvette and mixed with 500 μL of solution I (0.2 M NaOH, 40% (v/v) pyridine, 500 μM potassium ferricyanide). The UV-Vis spectrum (380-600 nm) of the oxidized Fe^{III} state was recorded immediately. Sodium dithionite (10 μL of 0.5 M solution in 0.5 M NaOH) was added and the UV-Vis spectrum of the reduced Fe^{II} state was recorded immediately. The pyridine hemochromagen concentration was determined using its Q bands, with the following extinction coefficients: P450s and globins: 34.7 mM^{-1} cm^{-1} at 557 nm; cytochromes *c*: 30.27 mM^{-1} cm^{-1} at 550 nm.

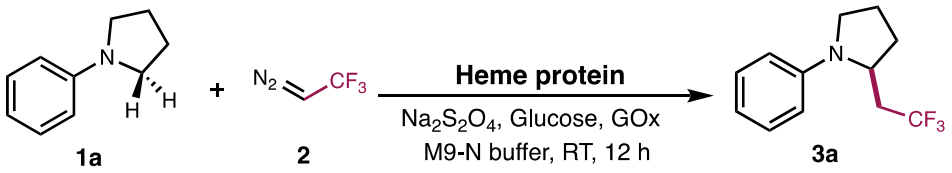
1.8 Biotransformation using clarified *E. coli* lysate. The lysate was placed in a sealed vial on ice and the headspace of the vial was purged with a stream of argon for at least 30 minutes. Enzymatic reactions were then set up in anaerobic chamber. To a 2 mL vial were added lysate (315 μL), a GOx oxygen depletion solution (20 μL of stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in M9-N buffer), *D*-glucose (20 μL of 250 mM stock solution in M9-N buffer), $Na_2S_2O_4$ (20 μL of 20 mM solution in M9-N), tertiary amine substrate (10 μL of 400 mM stock solution in EtOH) in the listed order. The perfluorodiazoalkane solution (15 μL of 600 mM stock solution in EtOH) kept in an ice-salt bath (-20 °C) was added at last. The reaction vials were then capped and shaken in the anaerobic chamber at room temperature and 700 rpm for 12 hours. After the completion of the reaction,

610 μL 1:1 ethylacetate/hexanes solution containing 0.66 mM 1,3,5-trimethoxybenzene internal standard was added to the vial. The resulting mixture was transferred to a 1.5 mL microcentrifuge tube, vigorously vortexed, and centrifuged (20,000 × g, 5 minutes) to completely separate the organic and aqueous layers. The organic layer was transferred to a vial equipped with an autosampler vial insert and analyzed by GC-MS. To prepare samples for enantioselectivity determination via normal-phase chiral HPLC, the organic solution in the vial was removed under reduced pressure and the resulting crude product was redissolved in hexanes.

1.9 Enzymatic preparative synthesis. 78.75 mL clarified lysate of *E. coli* harboring the P411-PFA variant was prepared as described in section **1.6**. The lysate was placed in a sealed vial on ice and the headspace was purged with a stream of argon for 30 minutes. To the reaction vial were added a GOx oxygen depletion solution (5 mL of stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in M9-N buffer), *D*-glucose (5 mL of 250 mM stock solution in M9-N buffer), $Na_2S_2O_4$ (5 mL of 20 mM solution in M9-N), and substrate **1** (2.5 mL of 400 mM stock solution in EtOH). 2,2,2-trifluoro-1-diazoethane **2** (3.75 mL of 600 mM stock solution in EtOH) kept in an ice-salt bath was added at last (-20 °C). The reaction vial was immediately capped and sealed with parafilm, removed from anaerobic chamber, and shaken at room temperature at 250 rpm for 12 hours. The reaction solution was then extracted with 100 mL 1:1 hexane/ethylacetate for three times. The combined organic layer was then washed with brine, dried over anhydrous $MgSO_4$, concentrated, and purified by flash chromatography.

II. Supplementary Tables and Figures

Table S1. Initial activity screening with heme and heme proteins.[a]



Catalyst	TTN	ee/%	Catalyst	TTN	ee/%
Blank M9-N buffer	NR	-	*A. pernix* protoglobin (*Ap*Pgb) W59G Y60Q	NR	-
Hemin[b]	**~ 2**	n.d.	*Ap*Pgb W59G Y60A	NR	-
R. marinus cytochrome c (*Rma* cyt c)	trace	n.d.	CYP119	trace	n.d.
Rma cyt c V75T M100D M103E	trace	n.d.	CYP119 C319S	NR	-
H. thermophilus cyt c	NR	-	$P450_{BM3}$	trace	n.d.
R. globiformis Cyt c WT	NR	-	$P411_{P\text{-}4}$ A82L	**40 ± 10**	n.d.
R. marinus nitric oxide dioxygenase *(Rma* NOD) Y32G	trace	n.d.	$P411_{CHA}$	**30 ± 10**	n.d.
Rma NOD Q52V	trace	n.d.	**P411-CH-C8**	**1250 ± 170**	**-12**[c]

[a] Experiments were performed using clarified *E. coli* lysate according to the protocol described in Section I. NR – no product was detected; n.d. – not determined.

[b] Experiments with hemin were performed using 5 μM hemin, 10 mM **1a**, 20 mM diazo substrate **2**, 10 mM $Na_2S_2O_4$.

[c] Enantiomeric excess compared to (*R*)-**3a**. The absolute configuration of (*R*)-**3a** was determined by X-ray crystallography (Section VIII).

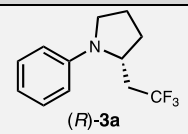
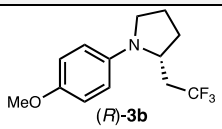
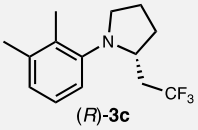
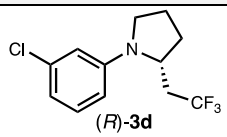
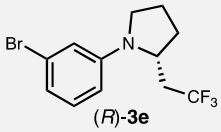
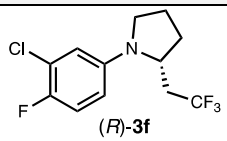
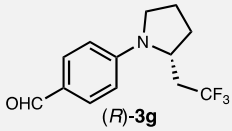
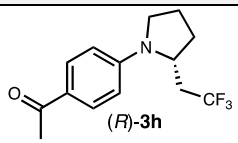
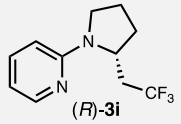
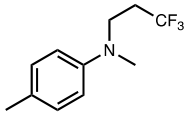
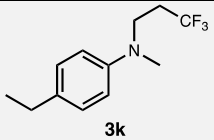
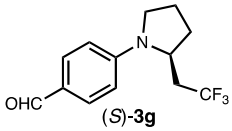
Table S2. Summary of directed evolution for enantioselective C–H fluoroalkylation.

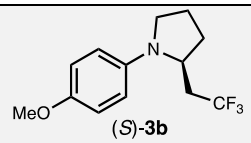
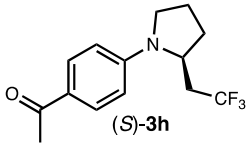
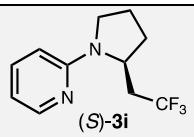
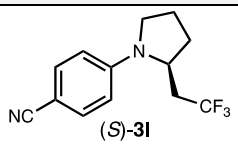
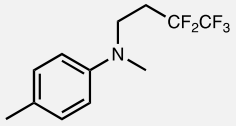
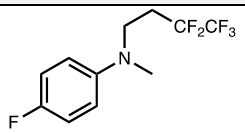
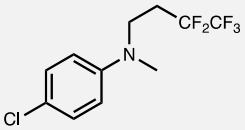
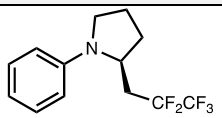
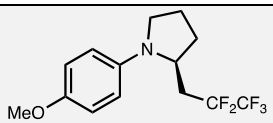
Gen.	Parent	Beneficial mutation identified	TTN	ee*
1	**P411-CH-C8**	T327V	1660 ± 40	28%
2	**P411-FA-B3** (P411-CH-C8 T327V)	E70T	1400 ± 150	32%
3	**P411-FA-B7** (P411-FA-B3 E70T)	L177M, R226T	1300 ± 130	80%
4	**P411-FA-C4** (P411-FA-B7 L177M R226T)	Y330V	1510 ± 240	88%
5	**P411-FA-E3** (P411-FA-C4 Y330V)	L401P	4070 ± 170	98%

* Enantiomeric excess (ee) of (*R*)-**3a**.

Experiments were performed using clarified *E. coli* lysate harboring the corresponding protein prepared according to the protocol described in Section I. Reactions were performed in triplicate. TTNs reported are the average of three experiments. Total turnovers (TTN) were defined as the amount of product divided by the total amount of expressed cytochrome P411 protein as determined by the hemochrome assay. Enantiomeric excess (ee) of **3a** was determined by normal phase chiral HPLC as mentioned in section VII. The absolute configuration of **3a** was determined by X-ray crystallography (Section VIII).

Table S3. Summary of product formation of P411-catalyzed enantioselective C–H fluoroalkylation.

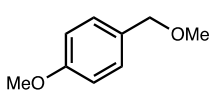
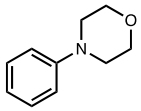
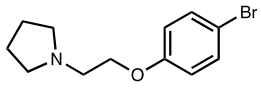
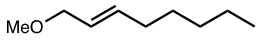
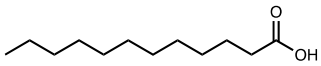
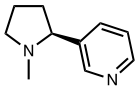
Product formed	Variant used	TTN	ee
 (*R*)-**3a**	P411-PFA	4070 ± 170	98%
 (*R*)-**3b**	P411-PFA	2100 ± 240	95%
 (*R*)-**3c**	P411-PFA	430 ± 40	>99%
 (*R*)-**3d**	P411-PFA	510 ± 70	>99%
 (*R*)-**3e**	P411-PFA	400 ± 60	>99%
 (*R*)-**3f**	P411-PFA	500 ± 20	92%
 (*R*)-**3g**	P411-PFA	690 ± 80	90%
 (*R*)-**3h**	P411-PFA	1440 ± 230	84%
 (*R*)-**3i**	P411-PFA	180 ± 10	88%
 **3j**	P411-PFA	1180 ± 80	-
 **3k**	P411-PFA	120 ± 10	-
 (*S*)-**3g**	P411-PFA-(*S*)	830 ± 50	94%*

 (*S*)-**3b**	P411-PFA-(*S*)	1620 ± 110	82%*
 (*S*)-**3h**	P411-PFA-(*S*)	1820 ± 70	74%*
 (*S*)-**3i**	P411-PFA-(*S*)	180 ± 20	72%*
 (*S*)-**3l**	P411-PFA-(*S*)	1400 ± 70	82%*
 **5a**	P411-PFA	440 ± 80	-
 **5b**	P411-PFA	520 ± 60	-
 **5c**	P411-PFA	250 ± 20	-
 (*S*)-**5d**	P411-PFA	460 ± 50	>99%*
 (*S*)-**5e**	P411-PFA	1550 ± 80	>99%*

* Enantiomeric excess (ee) of the (*S*)-enantiomer.

Experiments were performed using clarified *E. coli* lysate harboring the corresponding protein prepared according to the protocol described in Section I. Reactions were performed in triplicate. TTNs reported are the average of three experiments. Total turnovers (TTN) were defined as the amount of product divided by the total amount of expressed cytochrome P411 protein as determined by the hemochrome assay. Enantiomeric excess (ee) of the products were determined by normal phase chiral HPLC or chiral GC as mentioned in section VII.

Table S4. Additional substrates tested for P411-catalyzed C–H fluoroalkylation.

Benzylic C–H bond		**Other *N*-aryl amine**		***α*-Amino C–H bonds of aliphatic amine**	
					
1-methoxy-4-(methoxymethyl)benzene		*N*-phenylmorpholine		1-(2-(4-bromophenoxy)ethyl) pyrrolidine	
With **P411-PFA**	**40 ± 2 TTN**	With **P411-PFA**	Trace	With **P411-PFA**	NR
With variant **P411-FA-B7**	**440 ± 10 TTN**	With variant **P411-FA-C4 T226R Y330E**	**P/I* = 0.11**	With variant **P411-FA-E3**	**P/I* = 0.09**
Allylic C–H bond		**Native substrate of $P450_{BM3}$**		**Other**	
					
(*E*)-1-methoxyoct-2-ene		Lauric acid		(*S*)-nicotine	
With **P411-PFA**	NR	With **P411-PFA**	NR	With **P411-PFA**	NR

* P/I: Product versus Internal standard ratio. Product formation was analyzed by GC-MS only. The identity of the product was not confirmed by comparison with chemically synthesized reference compounds or through isolation and characterization. These preliminary results are noteworthy, but should not be used alone for drawing conclusions.

Experiments were performed using *E. coli* whole cell harboring the corresponding protein at OD_{600} = 30. Reactions were performed in triplicate. TTNs reported are the average of three experiments. Total turnovers (TTN) were defined as the amount of product divided by the total amount of expressed cytochrome P411 protein as determined by the hemochrome assay.

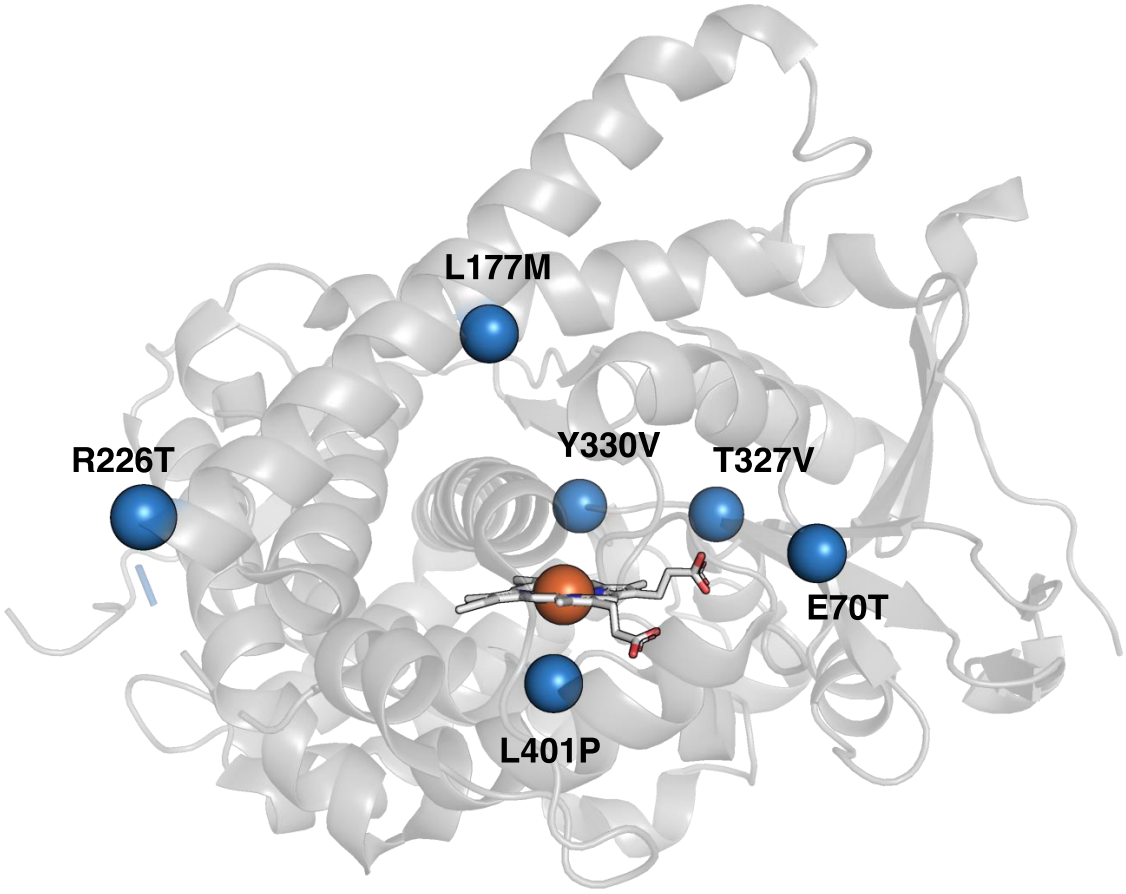
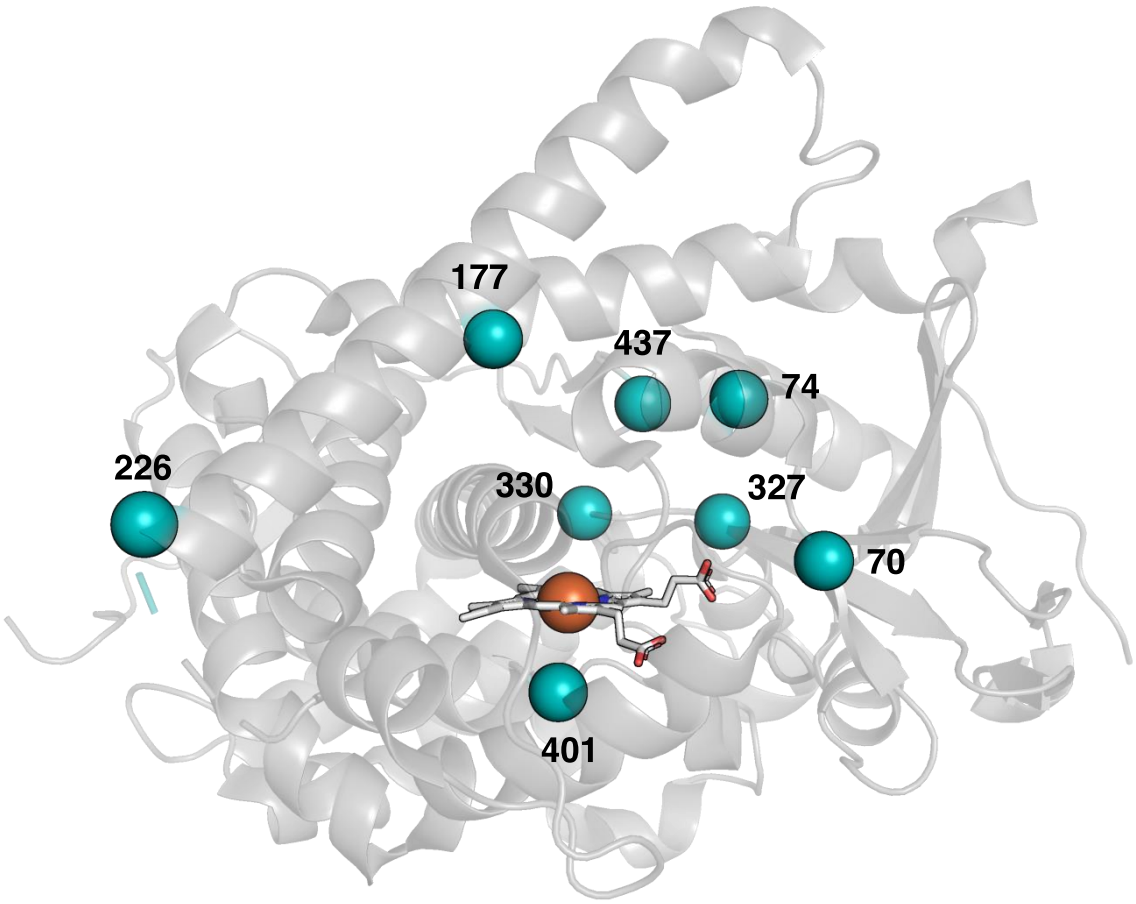


Figure S1. Structural visualization of amino acid positions which were mutated in the directed evolution trajectory of P411-CH-C8 to P411-PFA.



Position	70	74	177	226	327	330	401	437
P411-PFA	Thr	Gly	Met	Thr	Val	Val	Pro	Gln
P411-PFA-(*S*)	Glu	Pro	Leu	Arg	Thr	Tyr	Leu	Leu

Figure S2. Structural visualization of amino acid differences between P411-PFA-(*S*) and P411-PFA.

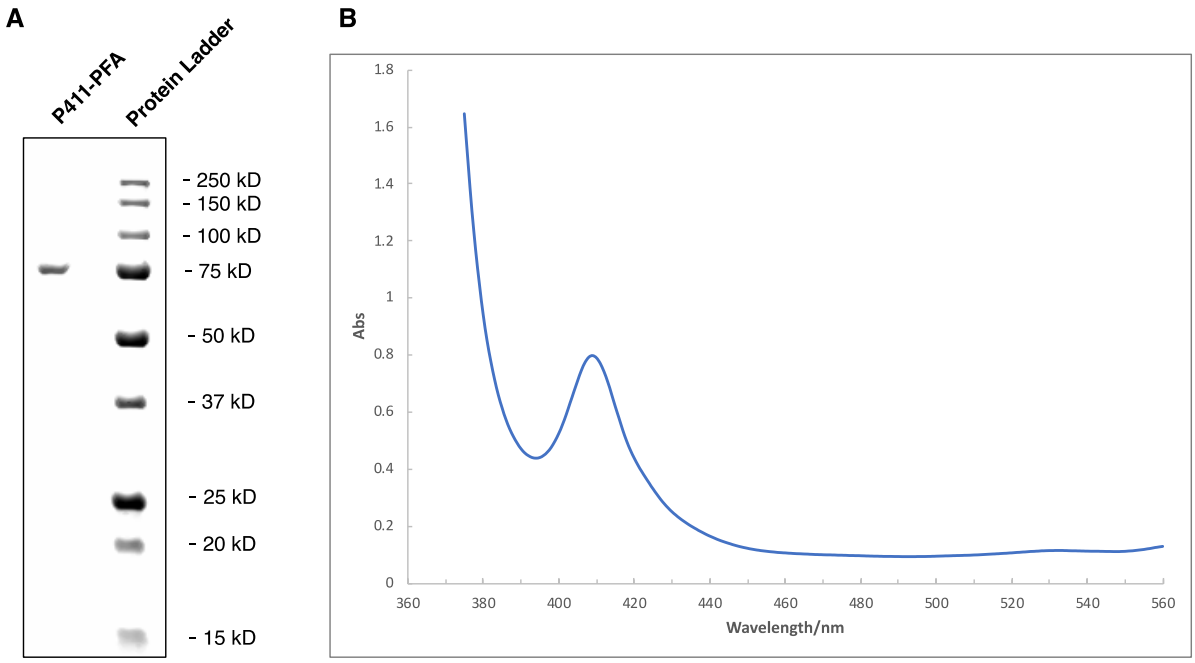
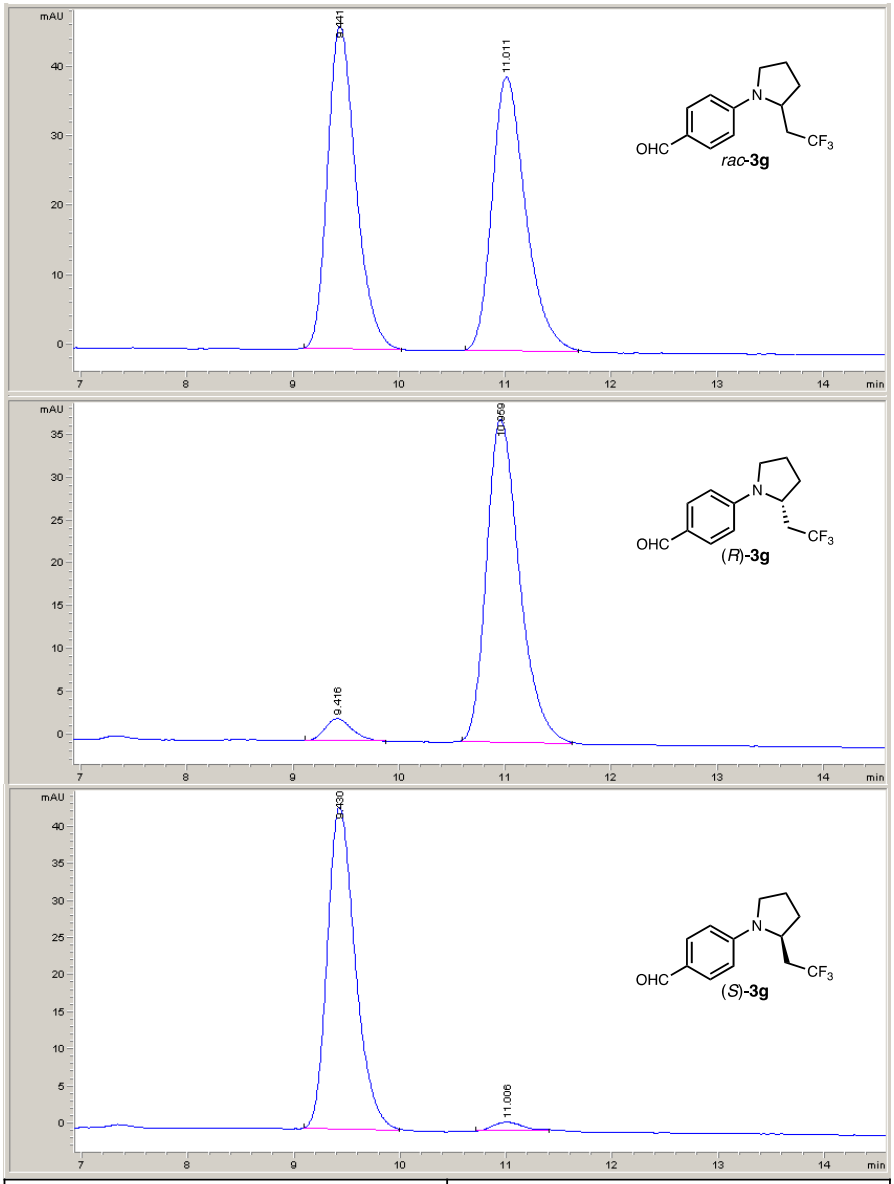


Figure S3. Characterization of P411-PFA protein. The final variant P411-PFA was purified by using a His-trap column. (**A**) SDS-PAGE gel of P411-PFA. The variant contains an engineered heme domain and the FMN domain of $P450_{BM3}$ (expected size, ~76 kDa); (**B**) UV-visible absorbance spectra of carbon monoxide-bound ferrous P411-PFA. The purified protein was suspended in KPi buffer (0.1 mM, pH = 8.0) with excess amount of NaS_2O_4. The solution was then purged with CO gas and the absorbance spectrum from 375 nm to 560 nm was immediately collected by a Shimadzu UV-1800 UV-Vis spectrophotometer. The maximum absorbance around 411 nm is consistent with the feature of a typical cytochrome P411 variant.[4]



rac-**3g**			Chiralpak IC (4.6 × 250 nm, 5µm), 30% IPA/hexanes, 1.5 mL/ min, 235 nm
Retention time (min)	Area (mAu)	Area%	
9.441	869	49.9	
11.011	874	50.1	
(*R*)-**3g** from P411-PFA			(*S*)-**3g** from P411-PFA-(*S*)
Retention time (min)	Area (mAu)	Area%	Retention time (min) / Area (mAu) / Area%
9.416	46	5.4	9.430 / 799 / 97.2
10.959	820	94.6	11.006 / 23 / 2.8

Figure S4. Chiral HPLC analysis of product **3g** obtained by P411-PFA and P411-PFA-(*S*).

III. Nucleotide and Amino Acid Sequences

The genes encoding the heme proteins shown below were cloned using Gibson assembly[1] into vector pET-22b(+) (Novagen) between restriction sites *Nde*I and *Xho*I in frame with a *C*-terminal 6xHis-tag.

DNA and amino acid sequences of **P411-CH-C8**:

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DNA and amino acid sequences of **P411-PFA**:

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DNA and amino acid sequences of **P411-PFA-(*S*)**:

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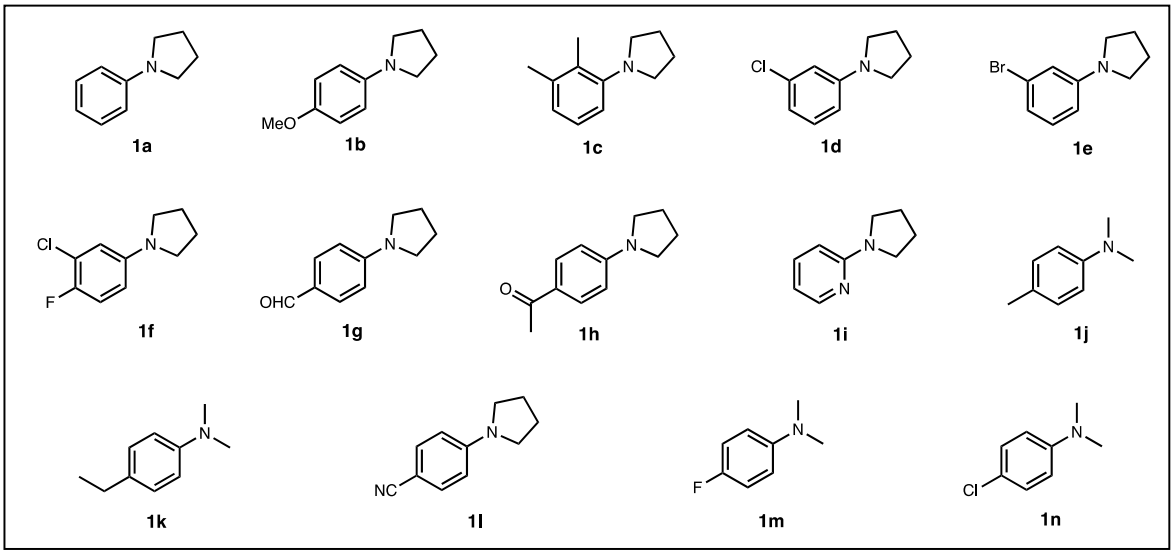
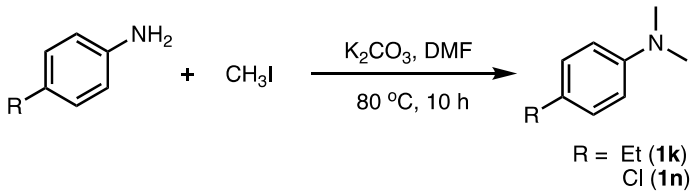
IV. Substrate Synthesis and Characterization[a]

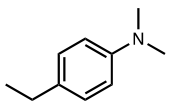


[a] **1a**-**1j**, **1i**, **1m** were obtained from commercial sources and were used as received.



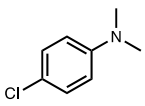
N, *N*-dimethyl anilines **1k** and **1n** were prepared according to a modified procedure reported by Shi[5] and McNally[6]. Namely, a mixture of aniline (5.0 mmol), iodomethane (11.0 mmol), and K_2CO_3 (11.0 mmol) in DMF (20 mL) was stirred at 80 °C for 10 h. The reaction was then cooled and diluted with EtOAc (100 mL) and H_2O (100 mL). The layers were separated and the organic layer was washed with brine (20 mL), dried over $MgSO_4$, filtered, concentrated, and purified by flash chromatography.

4-ethyl-*N*,*N*-dimethylaniline (1k)



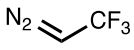
This compound is known.[7] **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.11 (d, *J* = 8.6 Hz, 2H), 6.74 (d, *J* = 8.6 Hz, 2H), 2.93 (s, 6H), 2.59 (q, *J* = 7.6 Hz, 2H), 1.23 (t, *J* = 7.6 Hz, 3H).

4-chloro-*N*,*N*-dimethylaniline (1n)



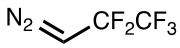
This compound is known.[8] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.17 (d, *J* = 9.1 Hz, 2H), 6.64 (d, *J* = 9.1 Hz, 2H), 2.93 (s, 6H).

2-diazo-1,1,1-trifluoroethane (2)



The stock solution of diazo compounds **2** was prepared using a modified procedure reported by Ma et al.[9] Namely, a solution of 610 mg $NaNO_2$ in 1 mL water was added slowly to a vigorously stirring solution of 1.08 g of trifluoroethylamine hydrochloride in 2 mL water at room temperature. The rapidly evolved yellow gas was carefully bubbled via PTFE tubing into 7 mL ethanol placed in a 10 mL vial and chilled on an ice-salt bath. After the cease of the gas bubbling (around 2-3 hours), the vial containing the 2-diazo-1,1,1-trifluoroethane ethanol solution was carefully removed from the ice-salt bath and stored in -20 ^{o}C freezer. The concentration of the stock solution was measured by ^{19}F NMR with fluorobenzene as internal standard.

3-diazo-1,1,1,2,2-pentafluoropropane (4)

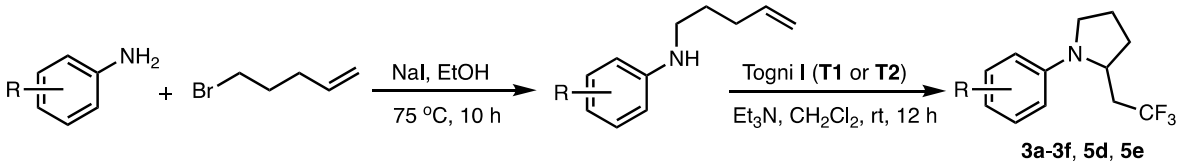


The stock solution of diazo compounds **4** was prepared by reacting $NaNO_2$ with 2,2,2,3,3-pentafluoropropylamine hydrochloride followed a procedure similar to the preparation of **2**. A slow stream of Ar was used to facilitate the transfer of **4** into the ethanol trapping solution. The concentration of the stock solution was measured by ^{19}F NMR with fluorobenzene as internal standard.

CAUTION: Diazo compounds are toxic and potentially explosive and should be handled with care in a well-ventilated hood.

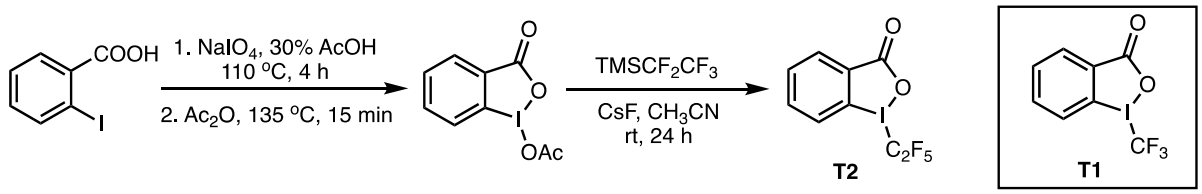
V. Synthesis and Characterization of Reference Compounds

General procedure A:



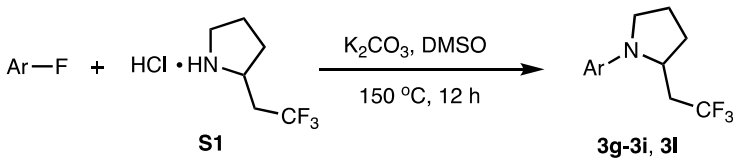
The synthesis of alkenylamine precursors was achieved with a procedure reported by Shen et al.[10]: To a suspension of NaI (0.1 mmol) in EtOH (25 mL), aniline (5 mmol), 5-bromopentene (3 mmol) was added, and the reaction was refluxed at 75 °C for 10 h. The resulting reaction mixture were concentrated and purified by flash chromatography.

Racemic reference compounds **3a-3f**, **5d**, **5e** were synthesized from the corresponding alkenylamines following the procedure reported by Kawamura et al.[11] Namely, a reaction vial charged with CuI (0.01 mmol) and Togni reagent I (0.30 mmol) was degassed and backfilled with argon for three times. A solution of Et_3N (0.04 mmol) in CH_2Cl_2 (0.5 mL) was then added, and the resulting suspension was stirred at room temperature. After 30 min, alkenylamine (0.20 mmol) was added and the reaction mixture was subsequently stirred for 12 h at room temperature. The crude reaction mixture was diluted with 5 mL CH_2Cl_2 and filtered. The collected filtrate was concentrated and purified by chromatography.



Togni reagent I (**T1**) is obtained from Sigma Aldrich and was used as received. Pentafluoroethyl Togni Reagent (**T2**) was synthesized according to the procedure reported by Li et al.[12].

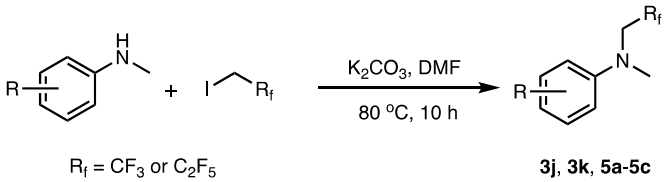
General procedure B:



Reference compounds **3g-3i**, **3l** were prepared from the S_NAr reaction between aryl fluorides and 2-(2,2,2-trifluoroethyl)pyrrolidine with the following protocol. To a suspension of K_2CO_3 (0.40 mmol, 55.2 mg) in DMSO (1.0 mL) was added 2-(2,2,2-trifluoroethyl)pyrrolidine hydrochloride **S1** (0.30 mmol, 57.0 mg, obtained from Enamine Ltd.). The reaction was

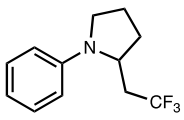
degassed and backfilled with argon for three times. The appropriate aryl fluoride (0.40 mmol) was then added, and the reaction was heated at 150 °C for 12 h. The reaction was cooled and thoroughly mixed with EtOAc (10 mL) and H_2O (10 mL). The layers were separated and the aqueous layer was further extracted with EtOAc (3 x 20 mL). The organic layers were combined, washed with brine (10 mL), dried over $MgSO_4$, filtered, concentrated. The obtained crude product was further purified by flash chromatography.

General Procedure C:



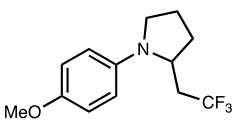
Reference compounds **3j**, **3k**, **5a-5c** were prepared from the *N*-fluoroalkylation of *N*-methylanilines with the following protocol. To a suspension of K_2CO_3 (1.1 mmol, 152 mg) in DMF (2.0 mL) was added the appropriate aniline (1.0 mmol). The reaction was degassed and backfilled with argon for three times. Polyfluoroalkyl iodide (0.40 mmol) was then added, and the reaction was heated at 150 °C for 12 h. The reaction was cooled to room temperature and thoroughly mixed with EtOAc (10 mL) and H_2O (10 mL). The layers were separated and the aqueous layer was further extracted with EtOAc (3 x 20 mL). The organic layers were combined, washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated. The obtained crude product was further purified by flash chromatography.

1-phenyl-2-(2,2,2-trifluoroethyl)pyrrolidine (3a)



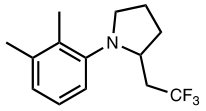
This compound is known.[11] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.35 – 7.18 (m, 2H), 6.72 (tt, *J* = 7.2, 1.1 Hz, 1H), 6.59 (dt, *J* = 7.2, 1.1 Hz, 2H), 4.04 (ddt, *J* = 10.5, 6.9, 1.7 Hz, 1H), 3.44 (ddd, *J* = 8.7, 7.1, 3.3 Hz, 1H), 3.17 (td, *J* = 8.6, 6.5 Hz, 1H), 2.62 – 2.44 (m, 1H), 1.97 – 2.14 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.2, 129.5, 126.3 (q, *J* = 277.7 Hz),116.4, 111.9, 52.6, 47.8, 36.7 (q, *J* = 25.4 Hz), 31.1, 23.0; **^{19}F NMR** (282 MHz, $CDCl_{3v}$) δ -63.9 (t, *J* = 11.2 Hz).

1-(4-methoxyphenyl)-2-(2,2,2-trifluoroethyl)pyrrolidine (3b)



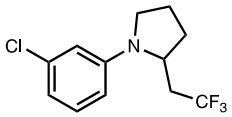
This compound is known.[11] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.92 – 6.83 (m, 2H), 6.55 (d, *J* = 8.5 Hz, 2H), 3.94 (dd, *J* = 10.5, 7.0 Hz, 1H), 3.76 (s, 3H), 3.41 (m, 1H), 3.13 (m, 1H), 2.63 – 2.40 (m, 1H), 2.21 – 1.95 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 151.3, 141.1, 126.4 (q, *J* = 277.9 Hz), 115.3, 112.9, 55.9, 53.0, 48.6, 37.0 (q, *J* = 24.9 Hz), 31.3, 23.2; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -63.9 (t, *J* = 11.2 Hz).

1-(2,3-dimethylphenyl)-2-(2,2,2-trifluoroethyl)pyrrolidine (3c)



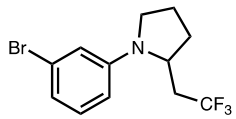
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (t, *J* = 7.7 Hz, 1H), 6.96 – 6.86 (m, 2H), 3.71 (dddd, *J* = 10.4, 8.5, 6.5, 2.6 Hz, 1H), 3.45 (ddd, *J* = 9.2, 7.6, 5.8 Hz, 1H), 2.64 (ddd, *J* = 9.2, 8.4, 5.9 Hz, 1H), 2.30 – 2.37 (m, 2H), 2.28 (s, 3H), 2.18 (s, 3H), 2.02 – 1.81 (m, 3H), 1.78 – 1.68 (m, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 147.6, 138.5, 133.1, 127.1 (q, *J* = 277.2 Hz), 126.2, 125.3, 117.2, 55.0 (q, *J* = 2.8 Hz), 54.1, 38.4 (q, *J* = 26.3 Hz), 32.1, 23.7, 21.0, 14.8; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -63.8 (t, *J* = 11.4 Hz); **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{14}H_{19}NF_3^+$: 258.1470, found: 258.1453.

1-(3-chlorophenyl)-2-(2,2,2-trifluoroethyl)pyrrolidine (3d)



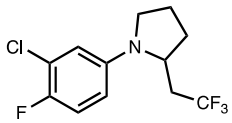
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (t, *J* = 8.1 Hz, 1H), 6.68 (ddd, *J* = 7.9, 2.0, 0.9 Hz, 1H), 6.55 (t, *J* = 2.2 Hz, 1H), 6.45 (ddd, *J* = 8.4, 2.5, 0.8 Hz, 1H), 4.01 (ddt, *J* = 10.3, 6.7, 1.8 Hz, 1H), 3.51 – 3.34 (m, 1H), 3.26 – 3.06 (m, 1H), 2.61 – 2.35 (m, 1H), 2.23 – 1.89 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 147.5, 135.7, 130.7, 126.5 (q, *J* = 277.7 Hz), 116.6, 112.2, 110.3, 53.0 (q, *J* = 3.1 Hz), 48.2, 36.9 (q, *J* = 25.7 Hz), 31.4, 23.3; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -63.9 (t, *J* = 11.1 Hz); **HRMS** (FAB) *m/z* $[M]^+$• calcd for $C_{12}H_{13}NClF_3^+$: 263.0689, found: 263.0691.

1-(3-bromophenyl)-2-(2,2,2-trifluoroethyl)pyrrolidine (3e)



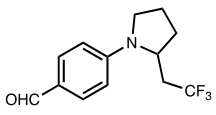
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (dd, *J* = 8.3, 7.9 Hz, 1H), 6.83 (ddd, *J* = 7.9, 1.8, 0.9 Hz, 1H), 6.71 (t, *J* = 2.2 Hz, 1H), 6.49 (ddd, *J* = 8.4, 2.5, 0.9 Hz, 1H), 4.09 – 3.95 (m, 1H), 3.41 (ddt, *J* = 8.8, 5.4, 1.7 Hz, 1H), 3.21 – 3.11 (m, 1H), 2.61 – 2.35 (m, 1H), 2.24 – 1.89 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 147.6, 131.0, 126.5 (q, *J* = 277.8 Hz), 124.0, 119.5, 115.1, 110.8, 53.0 (q, *J* = 3.0 Hz), 48.2, 36.9 (q, *J* = 25.7 Hz), 31.4, 23.3; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -63.8 (t, J = 11.1 Hz); **HRMS** (FAB) *m/z* $[M]^+$• calcd for $C_{12}H_{13}NBrF_3^+$: 307.0183, found: 307.0177.

1-(3-chloro-4-fluorophenyl)-2-(2,2,2-trifluoroethyl)pyrrolidine (3f)



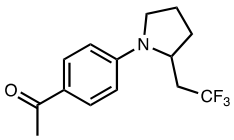
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (t, *J* = 8.9 Hz, 1H), 6.54 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.38 (dt, *J* = 9.1, 3.3 Hz, 1H), 3.94 (ddt, *J* = 10.3, 6.8, 1.7 Hz, 1H), 3.47 – 3.35 (m, 1H), 3.18 – 3.06 (m, 1H), 2.56 – 2.32 (m, 1H), 2.20 – 1.98 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 150.7 (d, *J* = 237.3 Hz), 143.6 (d, *J* = 1.8 Hz), 126.5 (q, *J* = 277.6 Hz), 121.7 (d, *J* = 18.3 Hz), 117.3 (d, *J* = 21.7 Hz), 113.3, 111.0 (d, *J* = 6.2 Hz), 53.4 (q, *J* = 3.0 Hz), 48.7, 37.0 (q, *J* = 25.7 Hz), 31.6, 23.4; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -63.9 (t, *J* = 11.0 Hz), -132.7; **HRMS** (FAB) *m/z* $[M]^+$• calcd for $C_{12}H_{12}NClF_4^+$: 281.0594, found: 281.0583

4-(2-(2,2,2-trifluoroethyl)pyrrolidin-1-yl)benzaldehyde (3g)



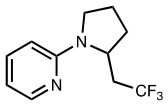
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.76 (d, *J* = 8.8 Hz, 2H), 6.61 (d, *J* = 8.8 Hz, 2H), 4.16 (ddt, *J* = 10.4, 3.9, 1.9 Hz, 1H), 3.52 (ddd, *J* = 10.9, 6.8, 2.1 Hz, 1H), 3.37 – 3.21 (m, 1H), 2.42 – 2.55 (m, 1H), 2.22 – 1.96 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 190.7, 150.8, 132.7, 126.3 (q, *J* = 277.7 Hz), 126.1, 111.9, 53.1 (q, *J* = 3.0 Hz), 48.1, 36.6 (q, *J* = 26.0 Hz), 31.2, 23.1; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -63.9 (t, *J* = 10.9 Hz); **HRMS** (FAB) *m/z* $[M + H]^+$ calcd for $C_{13}H_{15}ONF_3^+$: 258.1106, found: 258.1109.

1-(4-(2-(2,2,2-trifluoroethyl)pyrrolidin-1-yl)phenyl)ethan-1-one (3h)



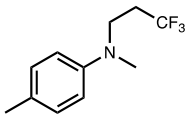
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 9.0 Hz, 2H), 6.56 (d, *J* = 9.0 Hz, 2H), 4.14 (ddt, *J* = 10.4, 5.3, 1.9 Hz, 1H), 3.51 (ddd, *J* = 8.4, 7.2, 3.4 Hz, 1H), 3.34 – 3.17 (m, 1H), 2.60 – 2.37 (m, 1H), 2.51 (s, 3H), 2.22 – 1.89 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 196.7, 149.7, 131.2, 126.3 (q, *J* = 277.7 Hz), 126.3, 111.4, 53.0 (q, *J* = 3.0 Hz), 48.0, 36.7 (q, *J* = 26.0 Hz), 31.3, 26.4, 23.1; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -63.9 (t, *J* = 10.9 Hz); **HRMS** (FAB) *m/z* $[M + H]^+$ calcd for $C_{14}H_{17}ONF_3^+$: 272.1262, found: 272.1267.

2-(2-(2,2,2-trifluoroethyl)pyrrolidin-1-yl)pyridine (3i)



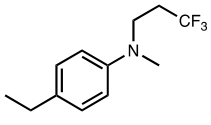
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (ddd, *J* = 5.0, 2.0, 0.9 Hz, 1H), 7.45 (ddd, *J* = 8.9, 7.1, 2.0 Hz, 1H), 6.57 (ddd, *J* = 7.1, 5.0, 0.9 Hz, 1H), 6.37 (dt, *J* = 8.6, 1.0 Hz, 1H), 4.47 – 4.23 (m, 1H), 3.52 (ddd, *J* = 9.8, 6.3, 5.1 Hz, 1H), 3.40 – 3.22 (m, 1H), 2.75 – 2.88 (m, 1H), 2.21 – 1.94 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.9, 148.7, 137.5, 126.7 (q, *J* = 277.8 Hz), 112.4, 106.9, 52.5 (q, *J* = 3.2 Hz), 47.4, 36.9 (q, *J* = 25.7 Hz), 30.8, 23.6; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -63.3 (t, *J* = 11.3 Hz); **HRMS** (FAB) *m/z* $[M + H]^+$ calcd for $C_{11}H_{14}N_2F_3^+$: 231.1109, found: 231.1124.

***N*,4-dimethyl-*N*-(3,3,3-trifluoropropyl)aniline (3j)**



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (dd, *J* = 8.9, 0.8 Hz, 2H), 6.67 (d, *J* = 8.6 Hz, 2H), 3.68 – 3.56 (m, 2H), 2.94 (s, 3H), 2.45 – 2.31 (m, 2H), 2.29 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.4, 130.3, 126.9, 126.9 (q, *J* = 276.9 Hz), 113.2, 46.4 (q, *J* = 3.5 Hz), 38.8, 30.8 (q, *J* = 27.1 Hz), 20.5; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -65.2 (t, *J* = 10.9 Hz); **HRMS** (FAB) *m/z* $[M]^{+\bullet}$ calcd for $C_{11}H_{14}NF_3^+$: 217.1078, found: 217.1085.

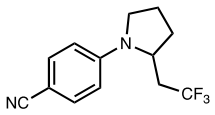
4-ethyl-*N*-methyl-*N*-(3,3,3-trifluoropropyl)aniline (3k)



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (dt, *J* = 8.8, 0.7 Hz, 2H), 6.68 (d, *J* = 8.8 Hz, 2H), 3.73 – 3.48 (m, 2H), 2.93 (s, 3H), 2.58 (q, *J* = 7.6 Hz, 2H), 2.45 – 2.26 (m, 2H), 1.22 (t, *J* = 7.6 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.6, 133.5, 129.1, 126.9 (q, *J* = 276.9 Hz), 113.1, 46.4 (q, *J* = 3.5 Hz), 38.9, 30.9 (q, *J* = 27.1 Hz), 28.1, 16.2; **^{19}F NMR** (282 MHz,

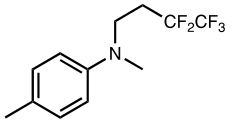
$CDCl_3$) δ -65.2 (d, *J* = 22.0 Hz); **HRMS** (FAB) *m/z* $[M]^+$• calcd for $C_{12}H_{16}NF_3^+$: 231.1298, found: 231.1326.

4-(2-(2,2,2-trifluoroethyl)pyrrolidin-1-yl)benzonitrile (3l)



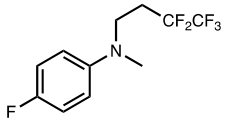
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 9.0 Hz, 2H), 6.54 (d, *J* = 8.9 Hz, 2H), 4.14 – 3.98 (m, 1H), 3.57 – 3.38 (m, 1H), 3.36 – 3.10 (m, 1H), 2.37 – 2.49 (m, 1H), 2.03 – 2.17 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 148.9, 134.1, 126.2 (q, *J* = 277.6 Hz), 120.8, 112.2, 98.5, 53.0 (q, *J* = 2.9 Hz), 48.0, 36.5 (q, *J* = 26.0 Hz), 31.3, 23.1; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -64.0 (t, *J* = 10.9 Hz); **HRMS** (FAB) *m/z* $[M + H]^+$ calcd for $C_{13}H_{14}N_2F_3^+$: 255.1104, found: 255.1103.

***N*,4-dimethyl-*N*-(3,3,4,4,4-pentafluorobutyl)aniline (5a)**



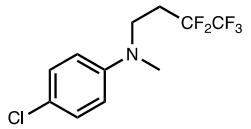
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (dd, *J* = 8.8, 0.8 Hz, 2H), 6.65 (d, *J* = 8.7 Hz, 2H), 3.88 – 3.50 (m, 2H), 2.92 (s, 3H), 2.34 – 2.16 (m, 2H), 2.27 (s, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.3, 130.3, 127.0, 118.1 (tq, *J* = 249.0, 36.2 Hz), 113.2, 45.1(t, *J* = 4.2 Hz), 38.8, 27.3 (t, *J* = 21.6 Hz), 20.6. The ^{13}C resonance corresponds to the -CF_3 group was not well resolved and the signal for this carbon is not reported; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -85.6, -118.3 (t, *J* = 18.6 Hz); **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{12}H_{15}NF_5^+$: 268.1125, found: 268.1123.

4-fluoro-*N*-methyl-*N*-(3,3,4,4,4-pentafluorobutyl)aniline (5b)



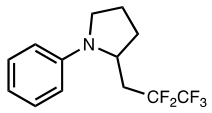
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (dd, *J* = 9.3, 8.3 Hz, 2H), 6.66 (dd, *J* = 9.2, 4.3 Hz, 2H), 3.70 – 3.57 (m, 2H), 2.91 (s, 3H), 2.41 – 2.12 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.2 (d, *J* = 236.3 Hz), 145.1 (d, *J* = 1.9 Hz), 119.3 (qt, *J* = 285.3, 36.1 Hz), 116.2 (d, *J* = 22.2 Hz), 115.8 (tq, *J* = 253.0, 38.2 Hz), 114.2 (d, *J* = 7.41 Hz), 45.6 (t, *J* = 4.4 Hz), 39.1, 27.4 (t, *J* = 21.7 Hz); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -85.6, -118.2 (t, *J* = 18.4 Hz), -128.1 (tt, *J* = 8.4, 4.2 Hz); **HRMS** (FAB) *m/z* $[M + H]^+$ calcd for $C_{11}H_{12}NF_6^+$: 272.0874, found: 272.0883.

4-chloro-*N*-methyl-*N*-(3,3,4,4,4-pentafluorobutyl)aniline (5c)



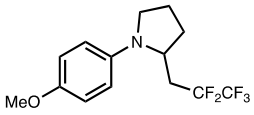
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, *J* = 9.1 Hz, 2H), 6.63 (d, *J* = 9.1 Hz, 2H), 3.79 – 3.54 (m, 2H), 2.94 (s, 3H), 2.46 – 2.14 (m, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.8, 129.6, 122.7, 114.0, 45.1 (t, *J* = 4.1 Hz), 38.8, 27.5 (t, *J* = 21.7 Hz). The ^{13}C resonance corresponds to the -CF_2CF_3 group was not well resolved and the signals for these carbons are not reported; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -85.6, -118.3 (t, *J* = 18.3 Hz); **HRMS** (FAB) *m/z* $[M]^+$• calcd for $C_{11}H_{11}NClF_5^+$: 287.0500, found: 287.0510.

2-(2,2,3,3,3-pentafluoropropyl)-1-phenylpyrrolidine (5d)



^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 – 7.21 (m, 2H), 6.73 (t, *J* = 7.3 Hz, 1H), 6.60 (d, *J* = 7.8 Hz, 2H), 4.23 – 4.11 (m, 1H), 3.56 – 3.36 (m, 1H), 3.18 (td, *J* = 8.8, 6.7 Hz, 1H), 2.36 – 2.54 (m, 1H), 2.20 – 1.82 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.4, 129.9, 116.8, 112.2, 51.9, 48.1, 33.5 (t, *J* = 20.3 Hz), 32.0, 23.4. The ^{13}C resonance corresponds to the -CF_2CF_3 group was not well resolved and the signals for these carbons are not reported; **^{19}F NMR** (282 MHz, , $CDCl_3$) δ -85.9, -100.7 – -126.1 (m); **HRMS** (FAB) *m/z* $[M]^{+}$• calcd for $C_{13}H_{14}NF_5^{+}$•: 279.1046, found: 279.1035.

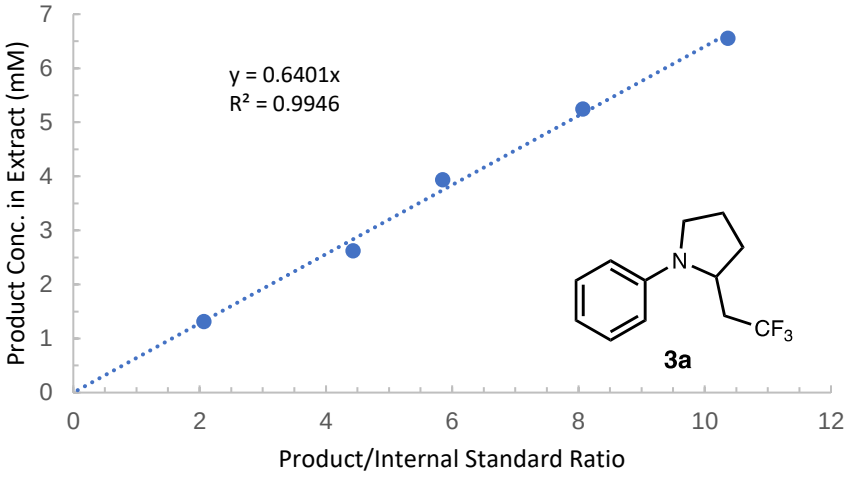
1-(4-methoxyphenyl)-2-(2,2,3,3,3-pentafluoropropyl)pyrrolidine (5e)

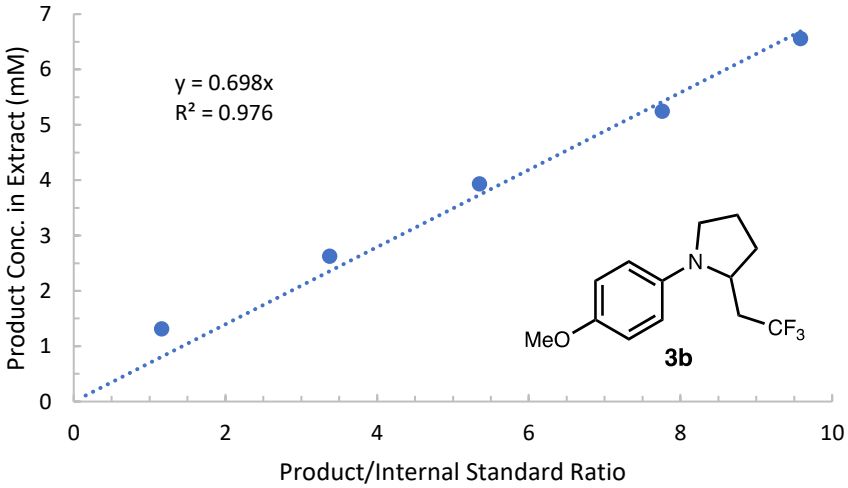


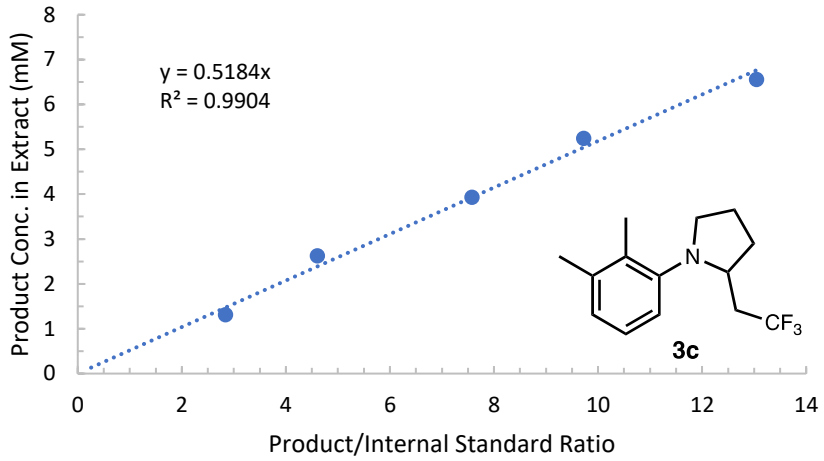
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (d, *J* = 9.0 Hz, 2H), 6.55 (d, *J* = 8.4 Hz, 2H), 4.09 (t, *J* = 8.6 Hz, 1H), 3.77 (s, 3H), 3.47 – 3.35 (m, 2H), 3.13 (q, *J* = 8.0 Hz, 1H), 2.60 – 2.33 (m, 1H), 2.21 – 2.10 (m, 1H), 2.12 – 1.81 (m, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 151.7, 141.3, 115.6, 113.1, 56.2, 52.3, 48.8, 33.9 (q, *J* = 20.8, 19.6 Hz), 32.2, 23.5. The ^{13}C resonance corresponds to the -CF_2CF_3 group was not well resolved and the signals for these carbons are not reported; **^{19}F NMR** (282 MHz, $CDCl_3$) δ 85.9, -117.5 (qdd, *J* = 180.0, 28.8, 9.8 Hz); **HRMS** (FAB) *m/z* $[M]^{+}$• calcd for $C_{14}H_{16}ONF_5^{+}$: 309.1152, found: 309.1162.

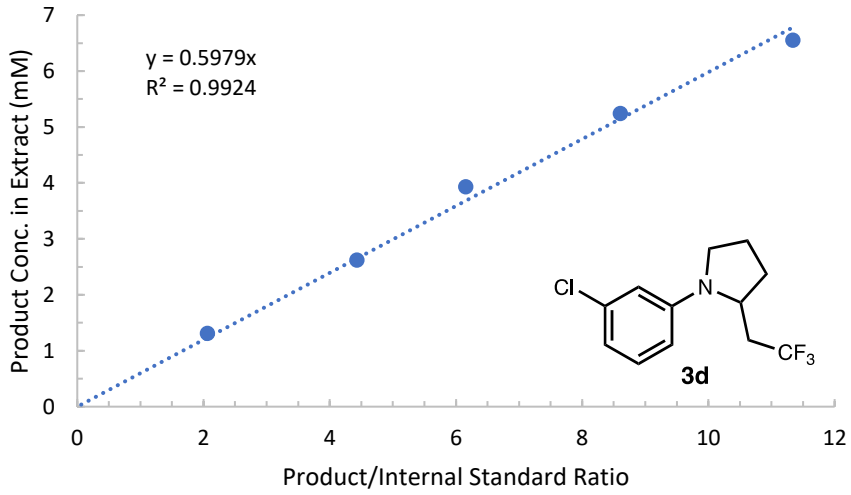
VI. Product GC-MS Calibration Curves

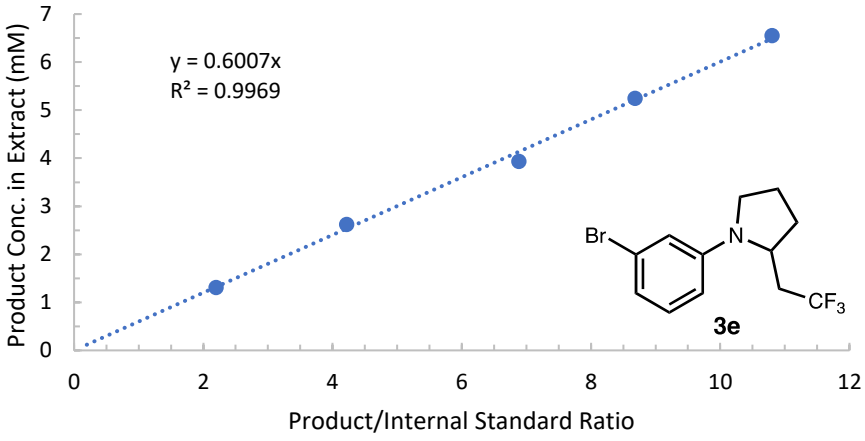
Product formation of **3a**-**3l**, **5a**-**5c** in enzymatic reactions was quantified by GC-MS based on standard curves. To determine the standard calibration curves, stock solutions of chemically synthesized authentic products were prepared at various concentrations (0.5 - 7 mM in 4:6 hexanes/EtOAc) with added internal standard 1,2,3-trimethoxybenzene (0.66 mM final concentration in the stock solutions). All data points represent the average of duplicate runs. The standard curves plot product concentration in mM (y-axis) against the ratio of product area to internal standard area on GC-MS (x-axis). For **5d** and **5e**, 15 analytical scale reactions were combined to measure the product formation and total turnover numbers by ^{19}F NMR with fluorobenzene as the internal standard.

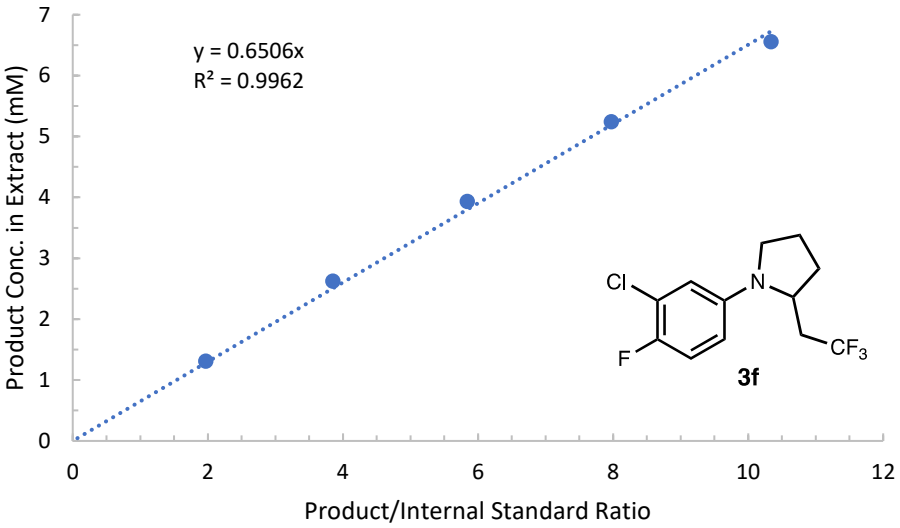


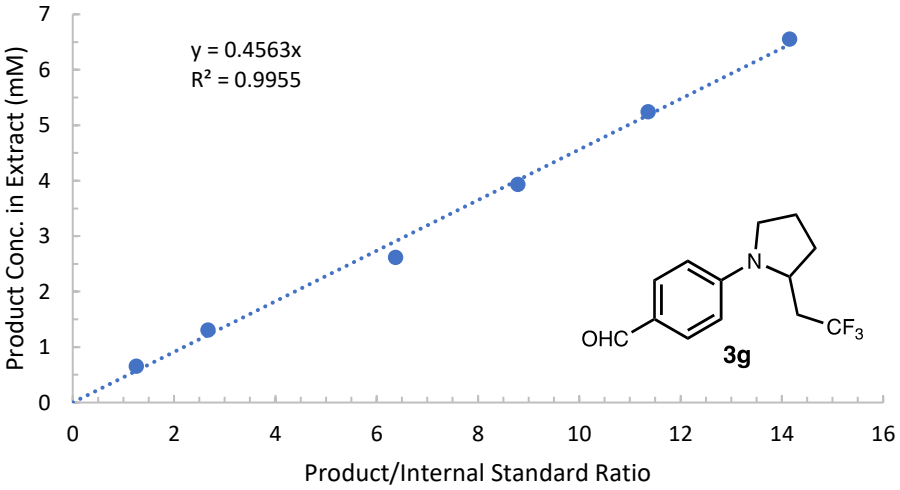


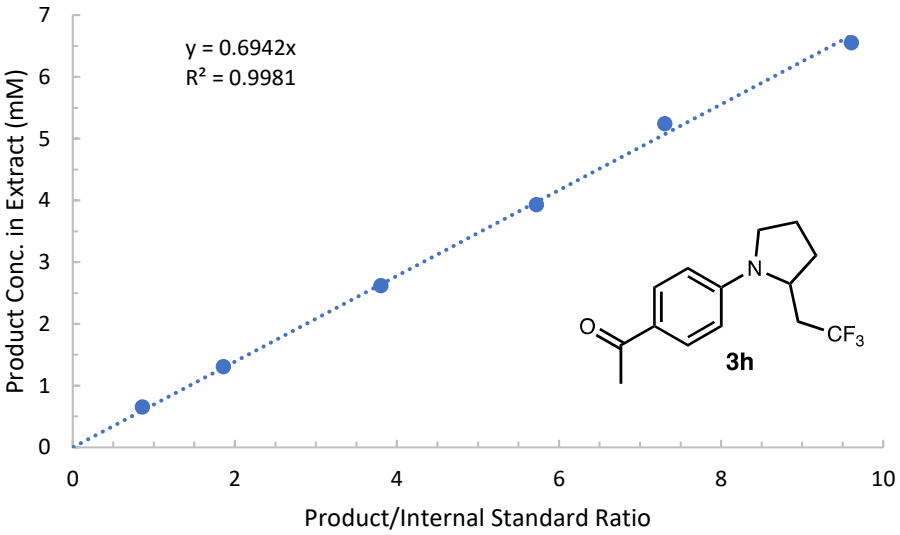

y = 0.5184x
R² = 0.9904
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
3c

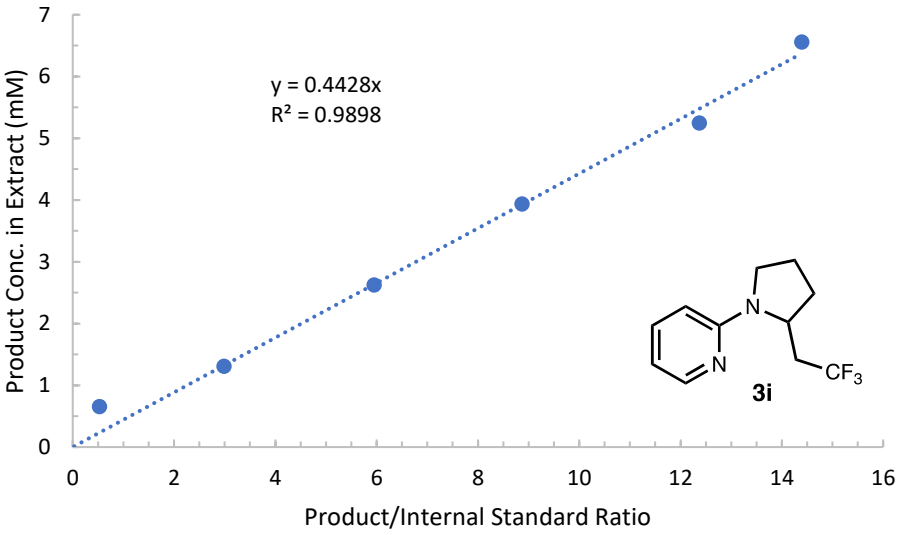

y = 0.5979x
R² = 0.9924
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
3d

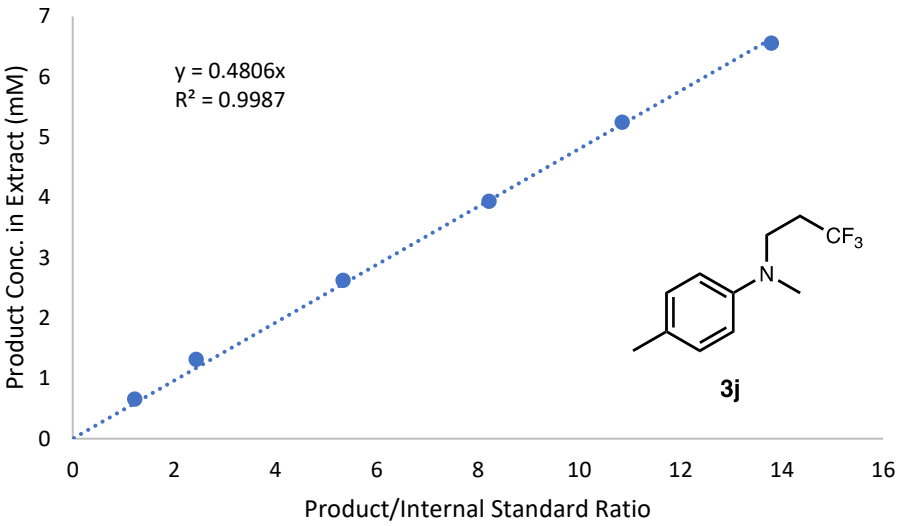

y = 0.6007x
R² = 0.9969
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
3e

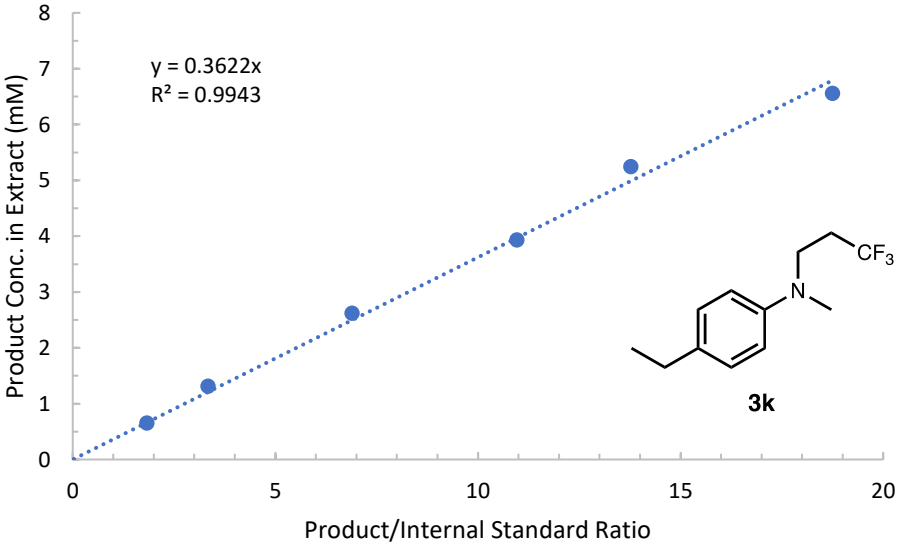

y = 0.6506x
R² = 0.9962
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
Cl
F
N
CF3
3f

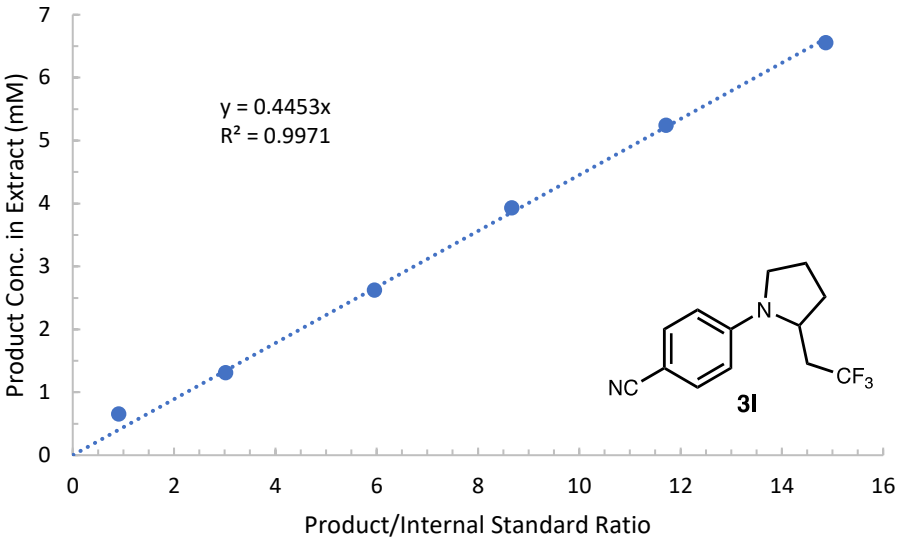

y = 0.4563x
R² = 0.9955
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
OHC
N
CF3
3g

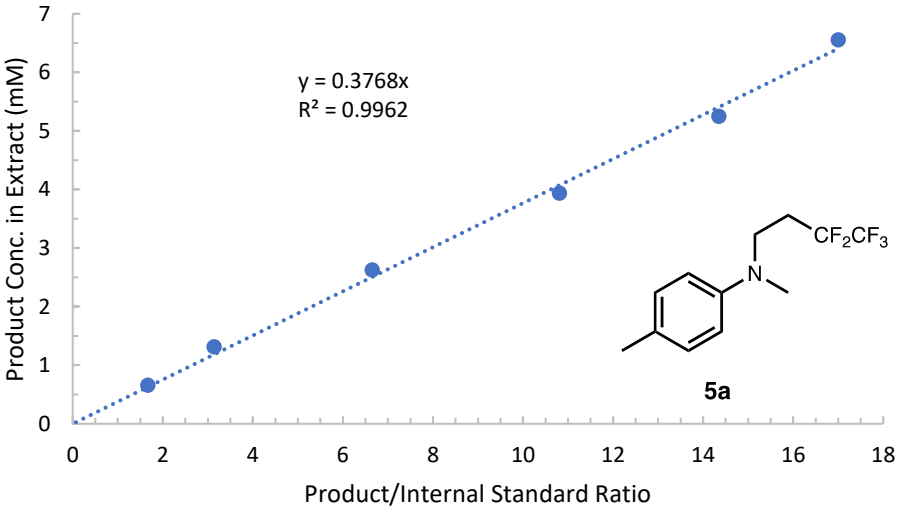

y = 0.6942x
R² = 0.9981
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
O
N
CF3
3h

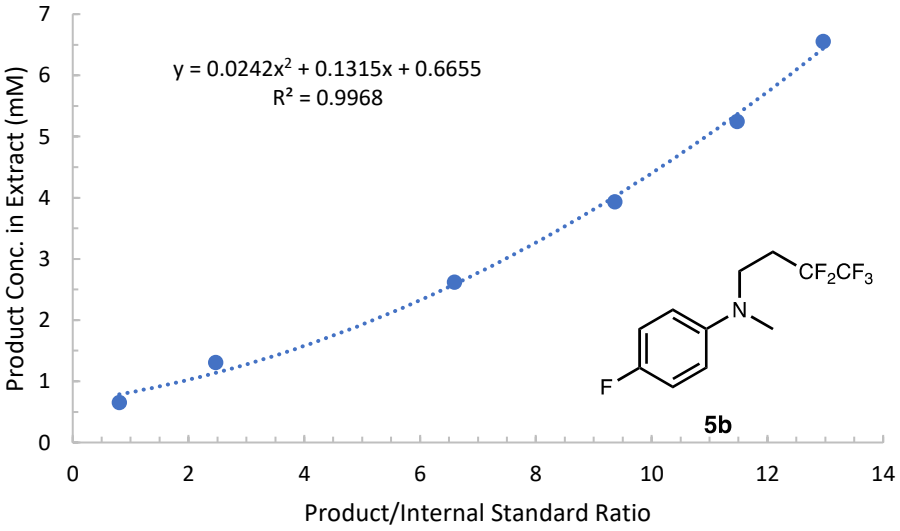

y = 0.4428x
R² = 0.9898
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16
N
N
CF3
3i

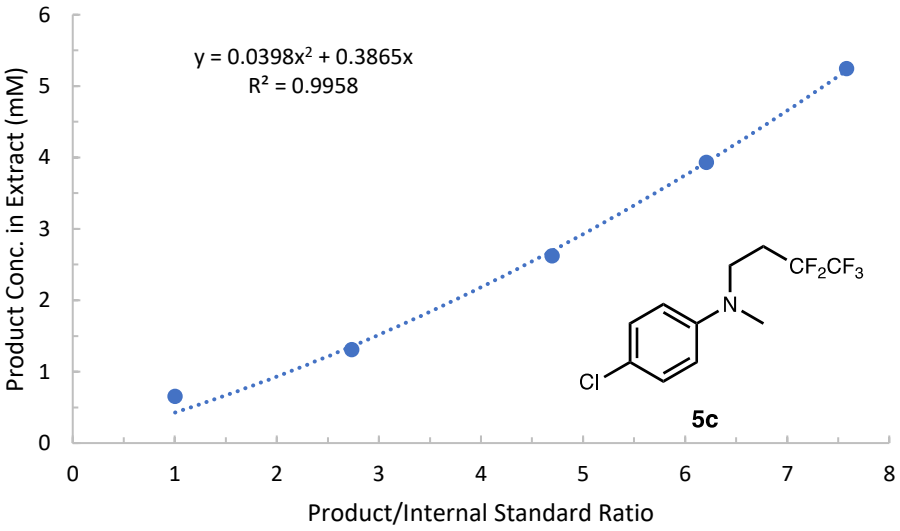

y = 0.4806x
R² = 0.9987
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16
CF3
N
3j


y = 0.3622x
R² = 0.9943
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
0
1
2
3
4
5
6
7
8
0
5
10
15
20
CF3
N
3k


y = 0.4453x
R² = 0.9971
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
NC
CF3
3l

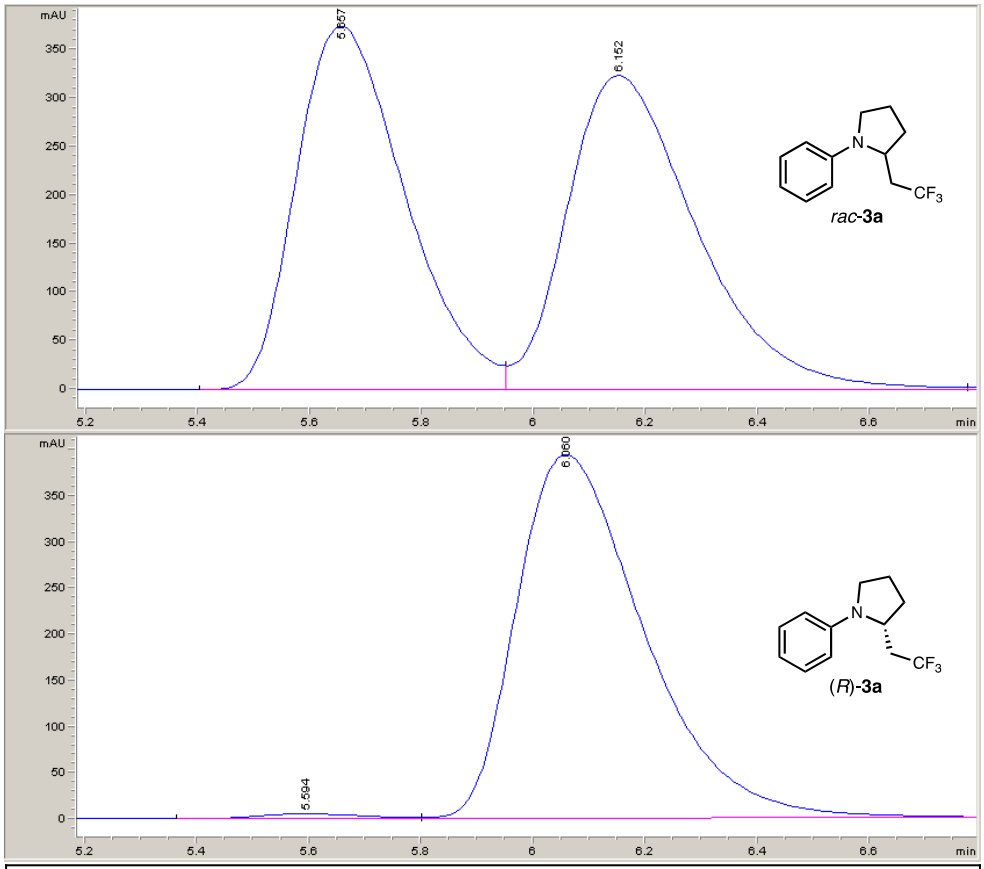

y = 0.3768x
R² = 0.9962
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
CF2CF3
5a


y = 0.0242x² + 0.1315x + 0.6655
R² = 0.9968
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
CF2CF3
F
5b

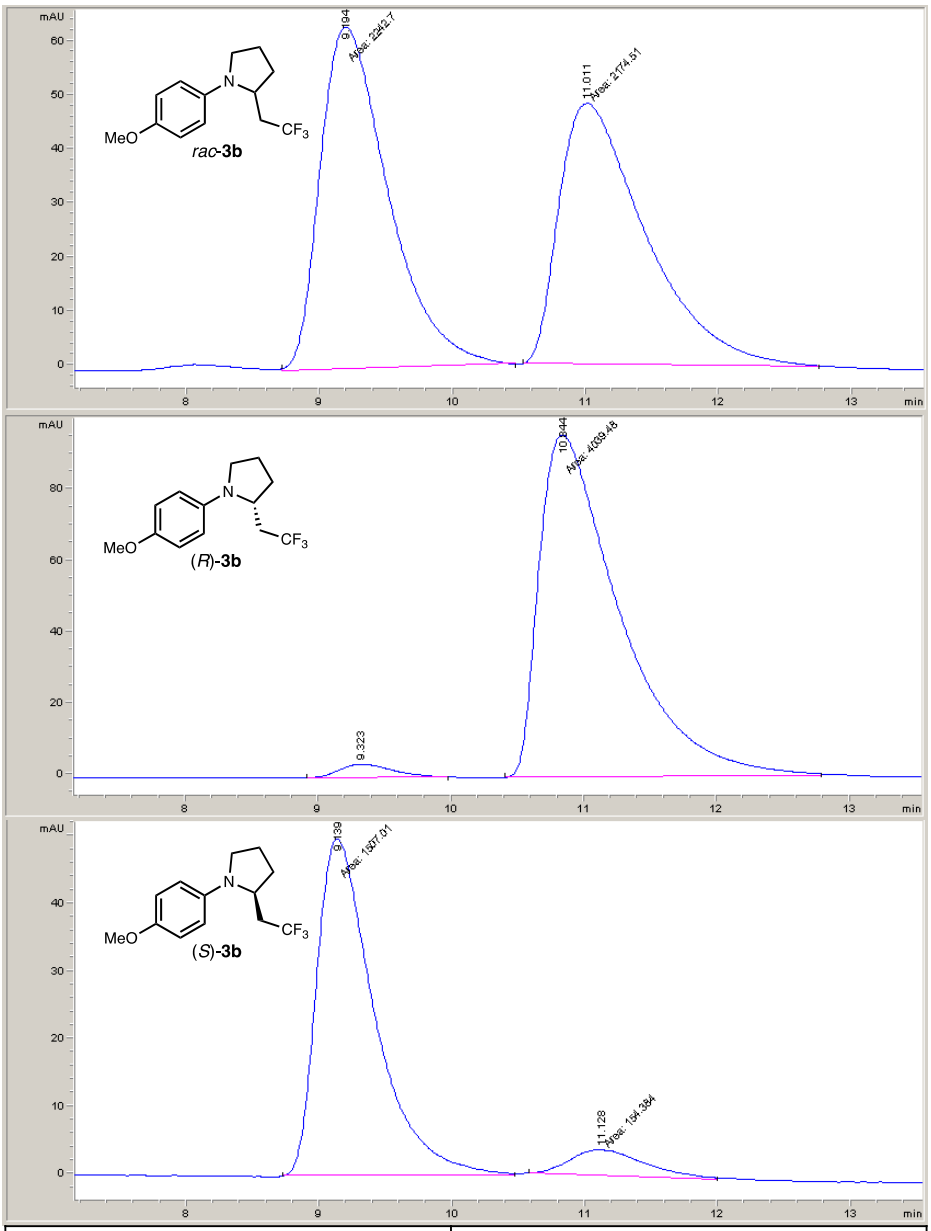

y = 0.0398x² + 0.3865x
R² = 0.9958
Product Conc. in Extract (mM)
Product/Internal Standard Ratio
0
1
2
3
4
5
6
7
8
CF2CF3
N
Cl
5c

VI. Determination of Enantioselectivity

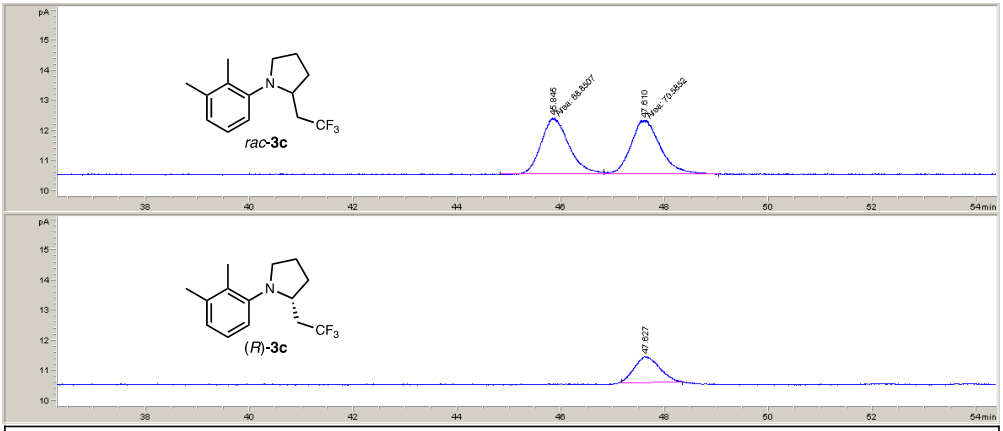
All e.e. values of enzymatically synthesized products were determined using normal-phase chiral HPLC or chiral GC. The absolute configuration of product **3a** synthesized by P411-PFA variant was determined to be *R* based on the X-ray crystallography data (see section VIII for details). Absolute configurations of other C–H trifluoroethylation products (**3b**-**3i**, **3l**) were inferred by analogy, assuming the facial selectivity of the trifluoroethyl diazo reagents from which these products were made remains the same as that of **3a**. The absolute configuration of product **5e** synthesized by P411-PFA variant were determined to be *S* based on the X-ray crystallography data (see section VIII for details). Absolute configuration of **5d** were inferred by analogy.



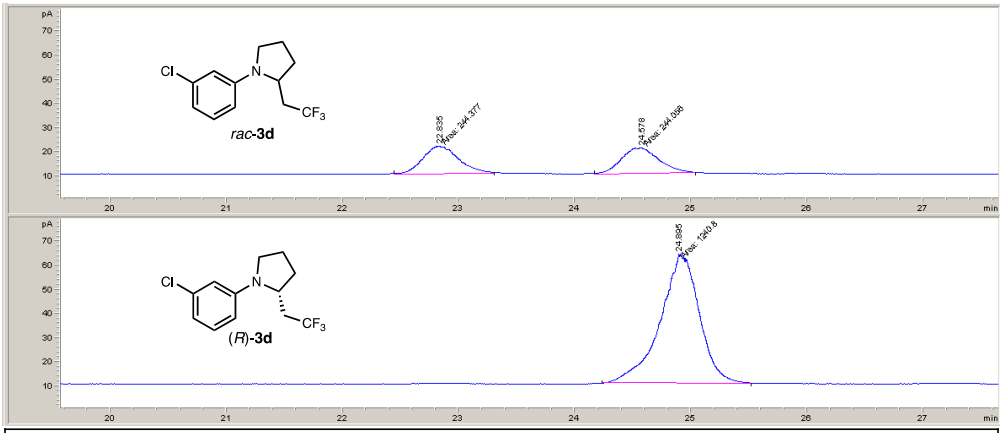
Chiralpak AD (4.6 × 250 nm, 5μm), 100% Hexanes, 1.0 mL/min, 254 nm					
rac-**3a**			(*R*)-**3a** from P411-PFA		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
5.657	5071	49.2	5.594	68	1.1
6.152	5228	50.8	6.060	6253	98.9



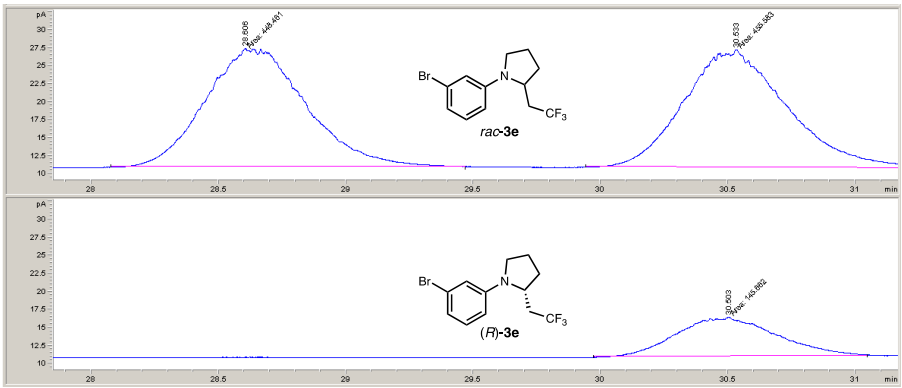
rac-**3b**			Chiralpak IA, 100% Hexanes, 1.5 mL/min, 254 nm		
Retention time (min)	Area (mAu)	Area%			
9.194	2246	50.2			
11.011	2225	49.8			
(*R*)-**3b**			(*S*)-**3b**		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
9.323	108	2.5	9.139	1511	91.0
10.844	4122	97.5	11.128	149	9.0



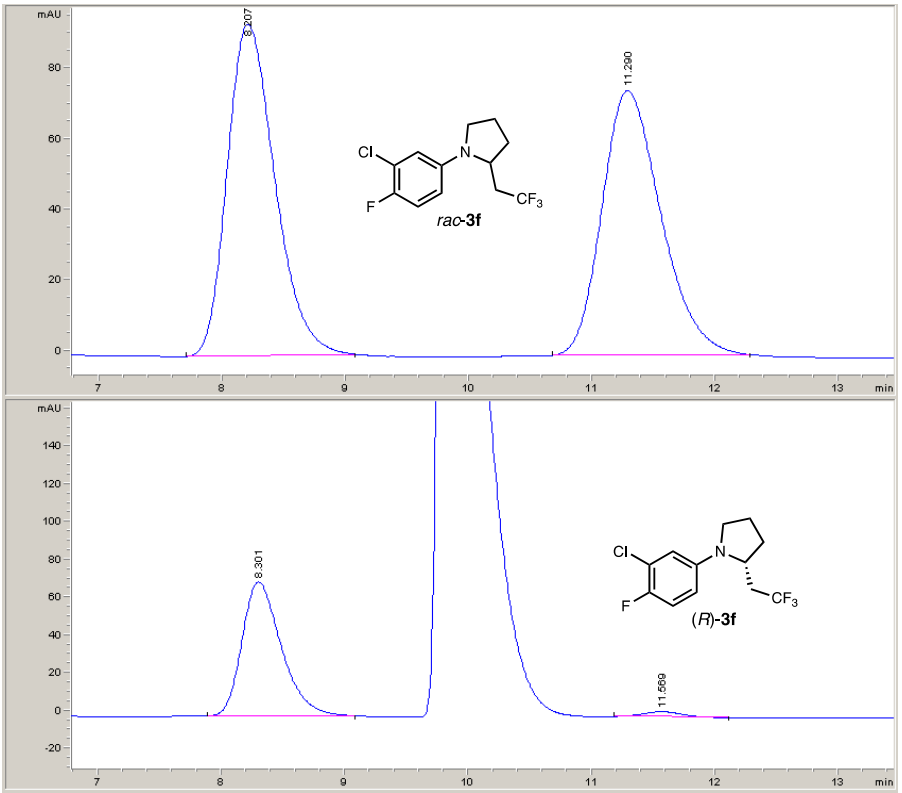
Astec CHIRALDEX G-TA (30 m × 0.25 mm × 0.12 µm), 100 °C					
rac-**3c**			(*R*)-**3c** from P411-PFA		
Retention time (min)	Area (pA)	Area%	Retention time (min)	Area (pA)	Area%
45.845	68.9	49.4	-	-	-
47.610	70.6	50.6	47.627	55.4	>99



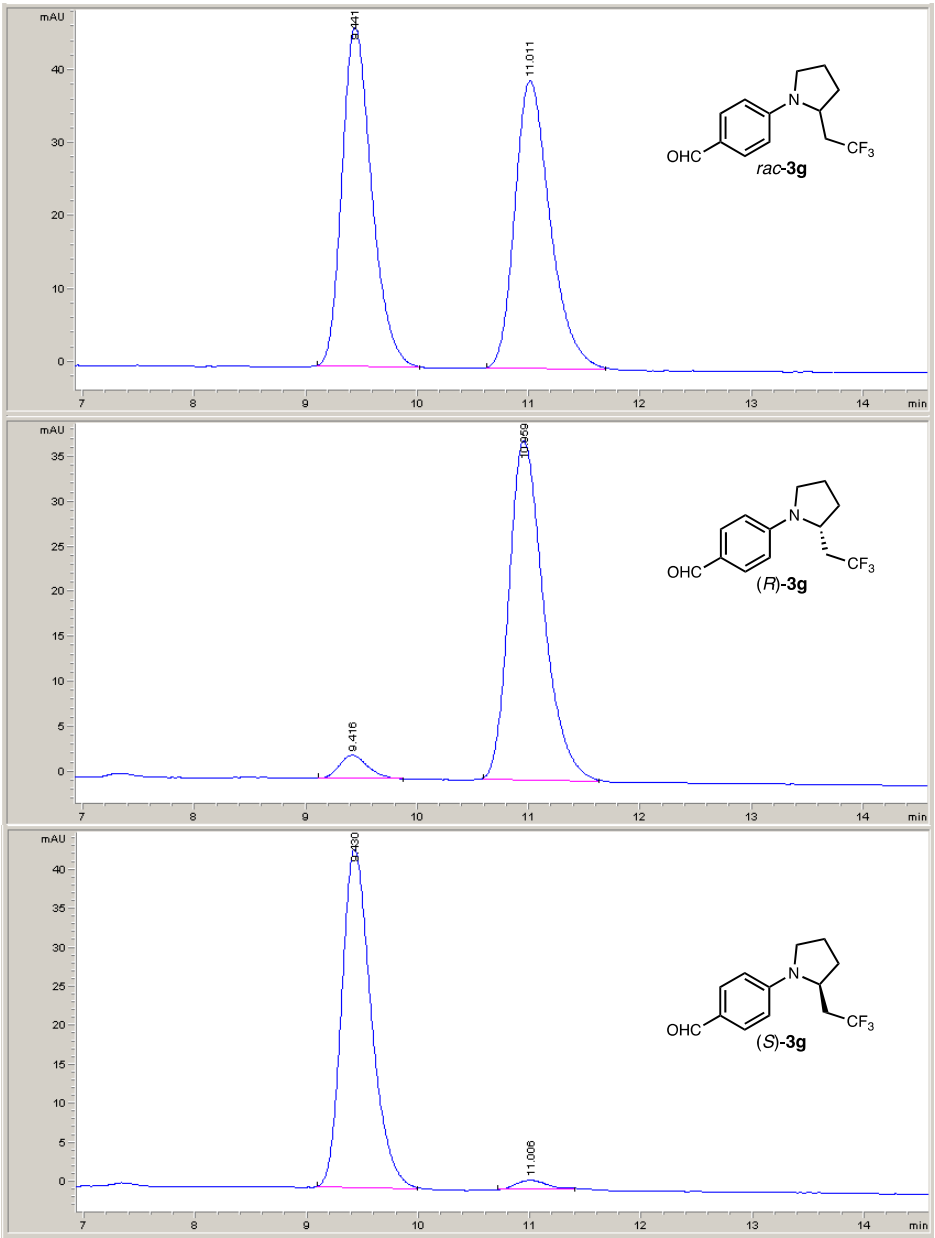
Astec CHIRALDEX G-TA (30 m × 0.25 mm × 0.12 µm), 140 °C					
rac-**3d**			(*R*)-**3d** from P411-PFA		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
22.835	244.4	50.0	-	-	-
24.578	244.1	50.0	24.895	1240.8	>99



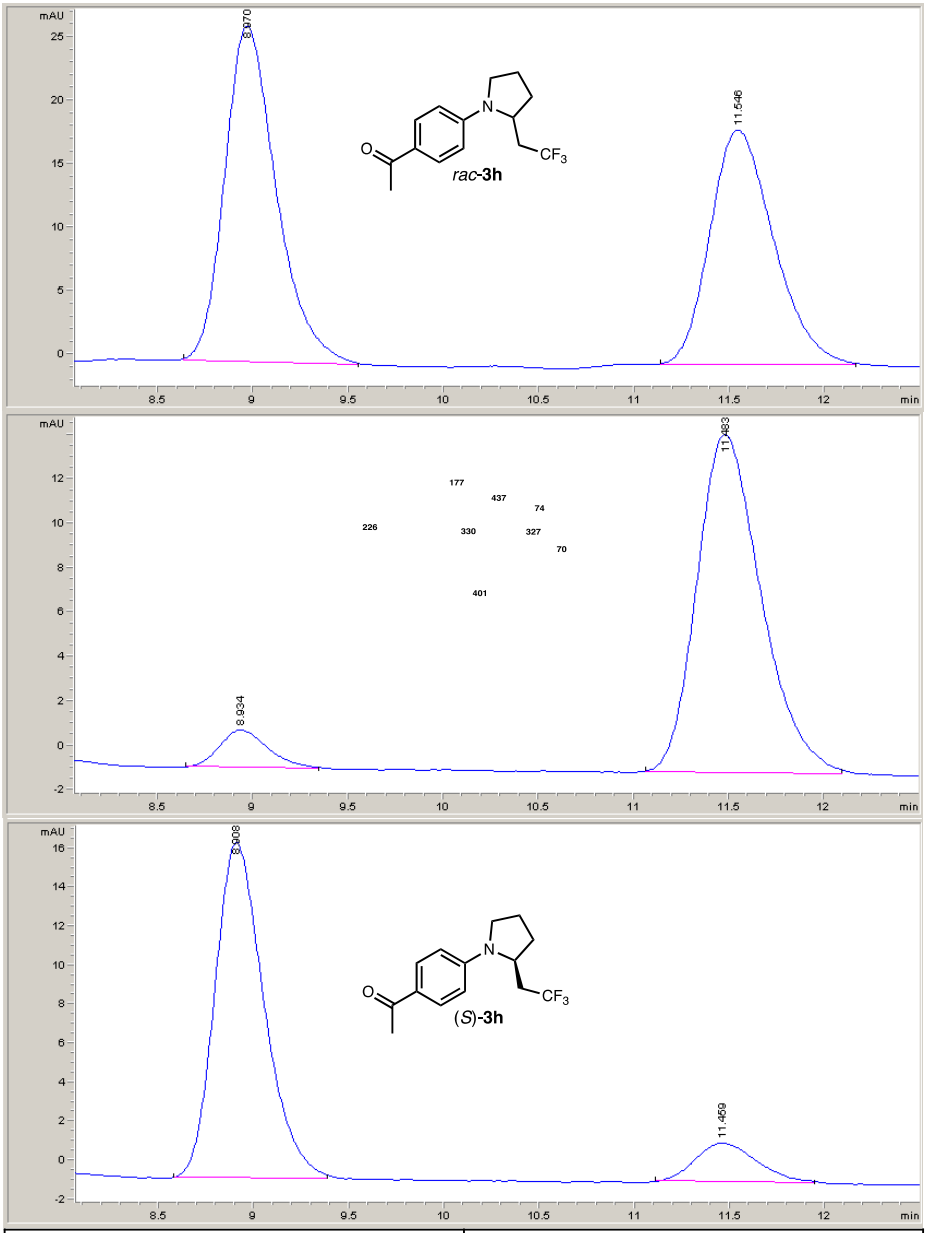
Astec CHIRALDEX G-TA (30 m × 0.25 mm × 0.12 µm), 145 °C					
rac-**3e**			(*R*)-**3e** from P411-PFA		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
28.606	448	49.6	-	-	-
30.533	456	50.4	30.503	146	>99



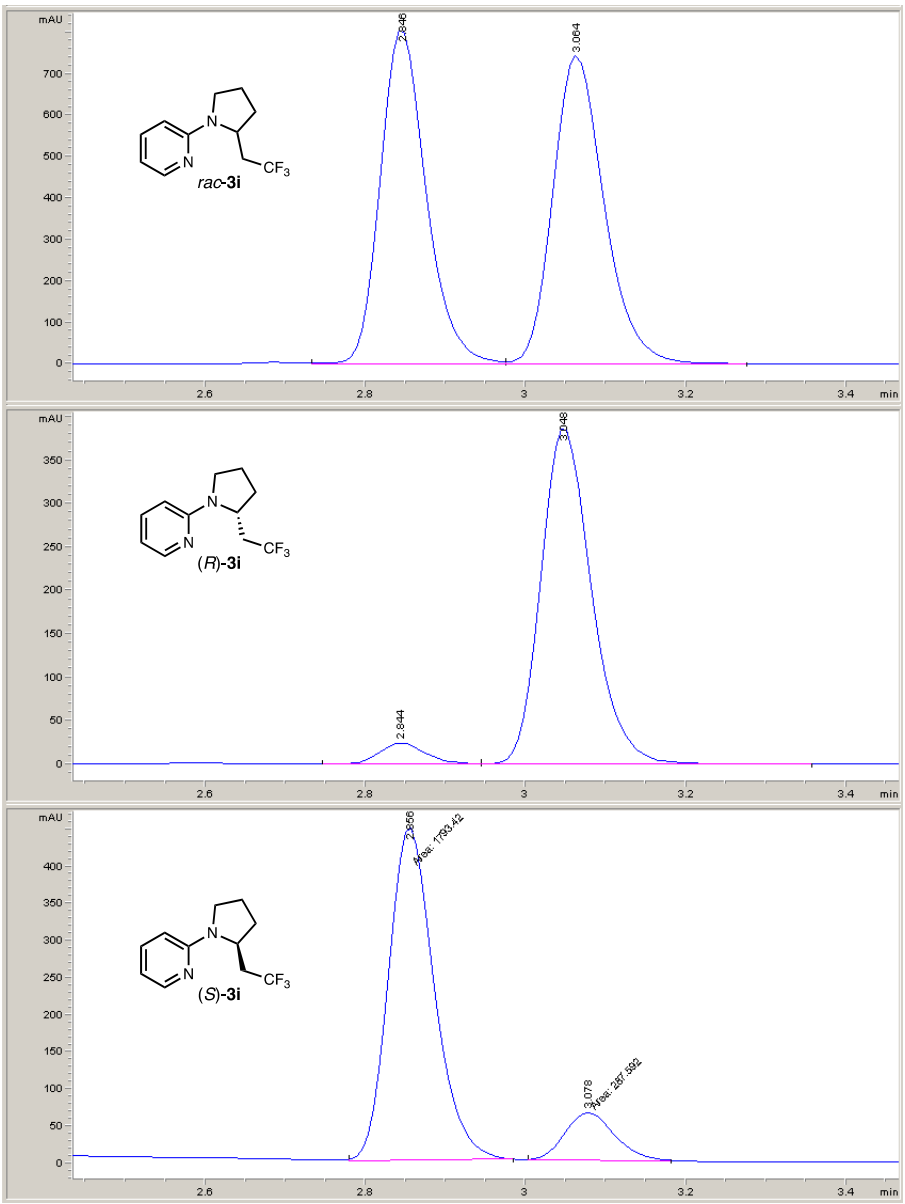
Chiralpak OJ-H (4.6 × 250 nm, 5µm), 100% Hexanes, 1.5 mL/min, 254 nm					
rac-**3f**			(*R*)-**3f** from P411-PFA		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
8.207	2537	50.3	8.301	1579	96.1
11.290	2508	49.7	11.569	64	3.9



rac-**3g**			Chiralpak IC (4.6 × 250 nm, 5µm), 30% IPA/hexanes, 1.5 mL/ min, 235 nm		
Retention time (min)	Area (mAu)	Area%			
9.441	869	49.9			
11.011	874	50.1			
(*R*)-**3g** from P411-PFA			(*S*)-**3g** from P411-PFA-(*S*)		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
9.416	46	5.4	9.430	799	97.2
10.959	820	94.6	11.006	23	2.8

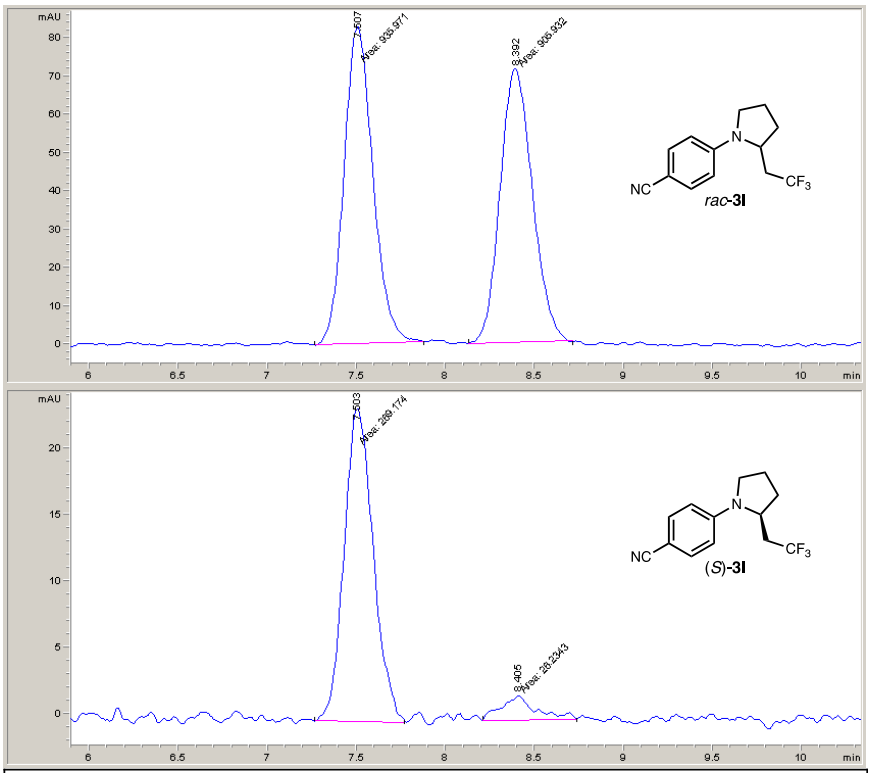


rac-**3h**			Chiralpak IC (4.6 × 250 nm, 5µm), 30% IPA/hexanes, 1.5 mL/ min, 235 nm
Retention time (min)	Area (mAu)	Area%	
8.970	478	52.7	
11.546	430	47.3	
(*R*)-**3h** from P411-PFA			(*S*)-**3h** from P411-PFA-(*S*)
Retention time (min)	Area (mAu)	Area%	Retention time (min) / Area (mAu) / Area%
8.934	30	7.8	8.908 / 309 / 87.1
11.483	357	92.2	11.459 / 46 / 12.9

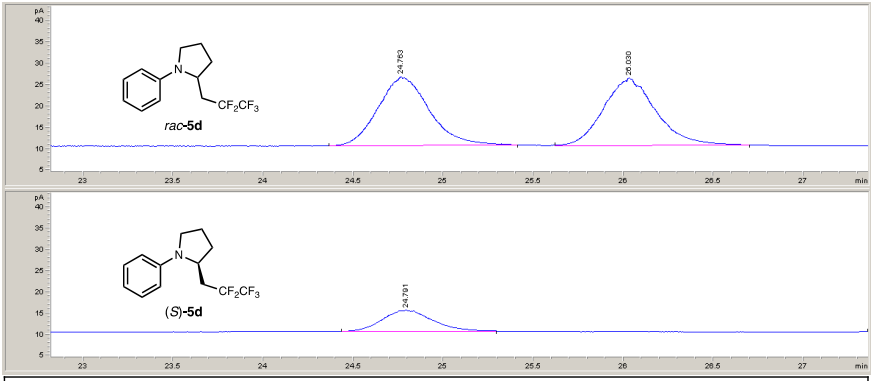


rac-**3i**			Chiralpak IC (4.6 × 250 nm, 5µm), 5% IPA/hexanes, 1.5 mL/min, 254 nm
Retention time (min)	Area (mAu)	Area%	
2.846	3219	49.6	
3.064	3270	50.4	

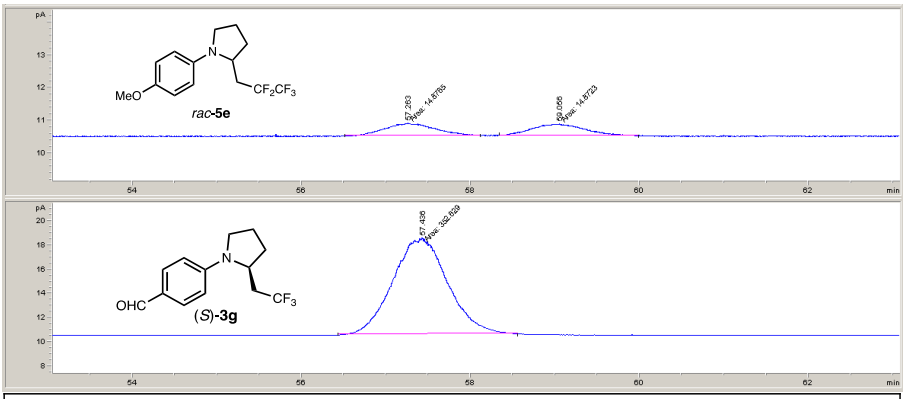
(*R*)-**3i** from P411-PFA			(*S*)-**3i** from P411-PFA-(*S*)		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
2.844	25	5.9	2.856	1793	86.2
3.048	389	94.1	3.078	288	13.8



Chiralpak IC (4.6 × 250 nm, 5μm), 30% IPA/hexanes, 1.5 mL/min, 254 nm					
rac-**3l**			(*S*)-**3l** from P411-PFA-(*S*)		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
7.507	936	50.8	7.503	269	91.2
8.392	906	49.2	8.405	26	8.8



Astec CHIRALDEX G-TA (30 m × 0.25 mm × 0.12 μm), 110 ℃					
rac-**5d**			(*S*)-**5d** from P411-PFA		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
24.763	317		24.791	95.6	>99
26.030	314		-	-	-



Astec CHIRALDEX G-TA (30 m × 0.25 mm × 0.12 µm), 120 ℃					
rac-**5e**			(*S*)-**5e** from P411-PFA		
Retention time (min)	Area (mAu)	Area%	Retention time (min)	Area (mAu)	Area%
57.263	14.8765	50.0	57.436	352.629	>99%
59.056	14.8723	50.0	-	-	-

VIII. X-Ray Crystallography and Assignments of Absolute Configuration

Crystal growth

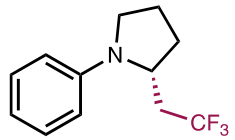
For product **3a**, 10 mg of pure enzymatic product was dissolved in 0.5 mL *n*-hexane in a 2 mL vial. The vial was loosely capped and left undisturbed at ambient temperature. After two days, the solvent was slowly evaporated and white, needle-like crystals were formed. The single crystals of product **5e** were obtained in a similar manner with *n*-pentane as the solvent.

Refinement details

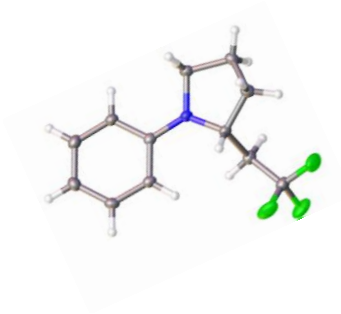
Crystals were mounted on a polyimide MiTeGen loop with STP Oil Treatment and placed under a nitrogen stream. Low temperature (100K) X-ray data were collected with a Bruker AXS D8 VENTURE KAPPA diffractometer running at 50 kV and 1mA (Cu K_α = 1.54178 Å; PHOTON II CPAD detector and Helios focusing multilayer mirror optics). All diffractometer manipulations, including data collection, integration, and scaling were carried out using the Bruker APEX3 software. An absorption correction was applied using SADABS. The space group was determined and the structure solved by intrinsic phasing using XT. Refinement was full-matrix least squares on F^2 using XL. All non-hydrogen atoms were refined using anisotropic displacement parameters. Hydrogen atoms were placed in idealized positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed at 1.2 times (1.5 times for methyl groups) the U_{eq} value of the bonded atom.

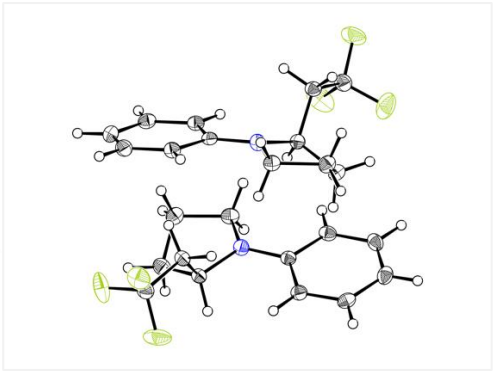
Special refinement details

Both compounds crystallize in the monoclinic space group $P2_1$. For compound **3a**, there are two molecules in the asymmetric unit; for compound **5e**, there is one.



3a





Identification code	v18648
Empirical formula	$C_{12}H_{14}F_3N$
Formula weight	229.24
Temperature/K	100.0
Crystal system	monoclinic
Space group	$P2_1$
a/Å	8.3312(11)
b/Å	17.574(2)
c/Å	8.3790(11)
α/°	90
β/°	116.975(5)
γ/°	90
Volume/Å^3	1093.3(3)
Z	4
ρ_{calc}g/cm^3	1.393
μ/mm^{-1}	1.008
F(000)	480.0
Crystal size/mm^3	0.28 × 0.21 × 0.15
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	10.066 to 161.532
Index ranges	-10 ≤ h ≤ 10, -22 ≤ k ≤ 22, -10 ≤ l ≤ 10
Reflections collected	50963
Independent reflections	4683 [R_{int} = 0.0463, R_{sigma} = 0.0198]
Data/restraints/parameters	4683/1/290
Goodness-of-fit on F^2	1.045
Final R indexes [I>=2σ (I)]	R_1 = 0.0279, wR_2 = 0.0705
Final R indexes [all data]	R_1 = 0.0279, wR_2 = 0.0705
Largest diff. peak/hole / e Å^{-3}	0.21/-0.19
Flack parameter	0.07(2)

Datablock: v18648

Bond precision: C-C = 0.0027 A Wavelength=1.54178

Cell: a=8.3312(11) b=17.574(2) c=8.3790(11)
alpha=90 beta=116.975(5) gamma=90
Temperature: 100 K

	Calculated	Reported
Volume	1093.3(2)	1093.3(3)
Space group	P 21	P 1 21 1
Hall group	P 2yb	P 2yb
Moiety formula	C12 H14 F3 N	C12 H14 F3 N
Sum formula	C12 H14 F3 N	C12 H14 F3 N
Mr	229.24	229.24
Dx,g cm-3	1.393	1.393
Z	4	4
Mu (mm-1)	1.008	1.008
F000	480.0	480.0
F000'	481.80	
h,k,lmax	10,22,10	10,22,10
Nref	4788[2474]	4683
Tmin,Tmax	0.776,0.860	0.785,1.000
Tmin'	0.754	

Correction method= # Reported T Limits: Tmin=0.785 Tmax=1.000
AbsCorr = MULTI-SCAN

Data completeness= 1.89/0.98 Theta(max)= 80.766

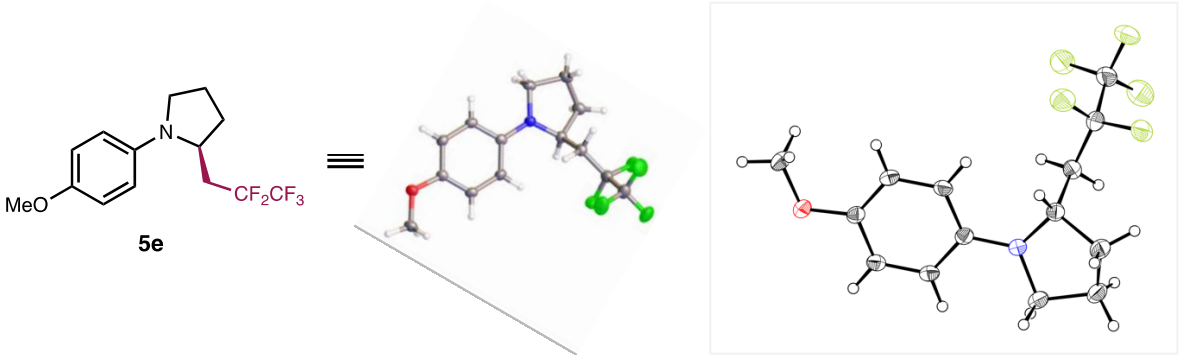
R(reflections)= 0.0279(4669) wR2(reflections)= 0.0705(4683)

S = 1.045 Npar= 290

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level G

PLAT033 ALERT 4 G	Flack x Value Deviates > 3.0 * sigma from Zero .	0.070 Note
PLAT791 ALERT 4 G	Model has Chirality at C10 (Chiral SPGR)	R Verify
PLAT791 ALERT 4 G	Model has Chirality at C10B (Chiral SPGR)	R Verify
PLAT883 ALERT 1 G	No Info/Value for _atom_sites_solution_primary .	Please Do !
PLAT912 ALERT 4 G	Missing # of FCF Reflections Above STh/L= 0.600	14 Note
PLAT913 ALERT 3 G	Missing # of Very Strong Reflections in FCF	1 Note
PLAT961 ALERT 5 G	Dataset Contains no Negative Intensities	Please Check
PLAT978 ALERT 2 G	Number C-C Bonds with Positive Residual Density.	17 Info



5e

Identification code	v18651
Empirical formula	$C_{14}H_{16}F_5NO$
Formula weight	309.28
Temperature/K	100
Crystal system	monoclinic
Space group	$P2_1$
a/Å	8.7849(11)
b/Å	5.4108(8)
c/Å	14.8752(19)
α/°	90
β/°	98.627(7)
γ/°	90
Volume/Å^3	699.07(16)
Z	2
ρ_{calc}g/cm^3	1.469
μ/mm^{-1}	1.212
F(000)	320.0
Crystal size/mm^3	0.24 × 0.23 × 0.12
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	6.01 to 158.618
Index ranges	$-10 \le h \le 11$, $-6 \le k \le 6$, $-18 \le l \le 18$
Reflections collected	22266
Independent reflections	2842 [R_{int} = 0.0414, R_{sigma} = 0.0219]
Data/restraints/parameters	2842/1/192
Goodness-of-fit on F^2	1.046
Final R indexes [I>=2σ (I)]	R_1 = 0.0276, wR_2 = 0.0694
Final R indexes [all data]	R_1 = 0.0279, wR_2 = 0.0696
Largest diff. peak/hole / e Å^{-3}	0.21/-0.14
Flack parameter	0.15(4)

Datablock: v18651

Bond precision: C-C = 0.0030 A Wavelength=1.54178

Cell: a=8.7849(11) b=5.4108(8) c=14.8752(19)
alpha=90 beta=98.627(7) gamma=90
Temperature: 100 K

	Calculated	Reported
Volume	699.07(16)	699.07(16)
Space group	P 21	P 1 21 1
Hall group	P 2yb	P 2yb
Moiety formula	C14 H16 F5 N O	C14 H16 F5 N O
Sum formula	C14 H16 F5 N O	C14 H16 F5 N O
Mr	309.28	309.28
Dx,g cm-3	1.469	1.469
Z	2	2
Mu (mm-1)	1.212	1.212
F000	320.0	320.0
F000'	321.35	
h,k,lmax	11,6,18	11,6,18
Nref	3035[1682]	2842
Tmin,Tmax	0.785,0.865	0.645,1.000
Tmin'	0.712	

Correction method= # Reported T Limits: Tmin=0.645 Tmax=1.000
AbsCorr = MULTI-SCAN

Data completeness= 1.69/0.94 Theta(max)= 79.309

R(reflections)= 0.0276(2809) wR2(reflections)= 0.0696(2842)

S = 1.046 Npar= 192

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level C

PLAT911_ALERT_3_C	Missing FCF Refl Between Thmin & STh/L=	0.600	21 Report
PLAT915_ALERT_3_C	No Flack x Check Done: Low Friedel Pair Coverage		90 %

Alert level G

PLAT033_ALERT_4_G	Flack x Value Deviates > 3.0 * sigma from Zero .	0.150	Note
PLAT242_ALERT_2_G	Low 'MainMol' Ueq as Compared to Neighbors of	C14	Check
PLAT791_ALERT_4_G	Model has Chirality at C11 (Chiral SPGR)	S	Verify
PLAT883_ALERT_1_G	No Info/Value for _atom_sites_solution_primary .	Please	Do !
PLAT912_ALERT_4_G	Missing # of FCF Reflections Above STh/L= 0.600	35	Note
PLAT978_ALERT_2_G	Number C-C Bonds with Positive Residual Density.	10	Info

VIII. Supplemental References

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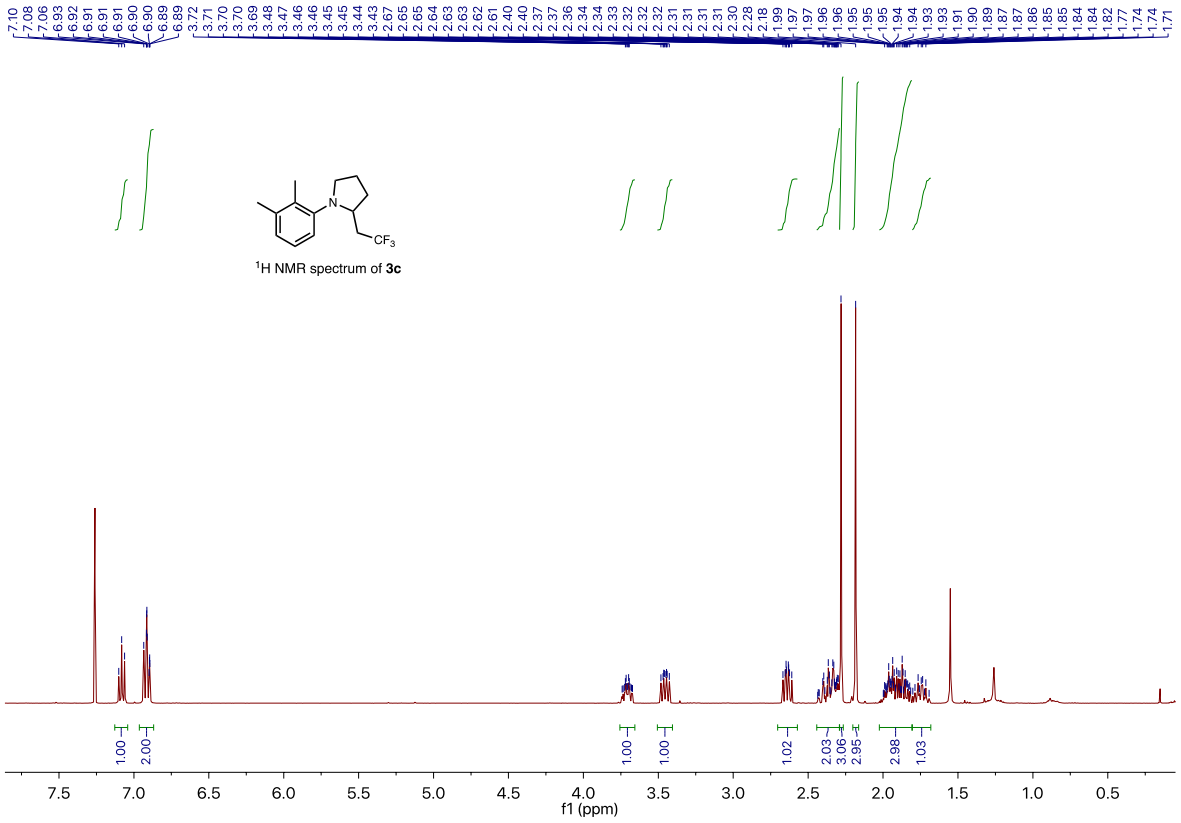
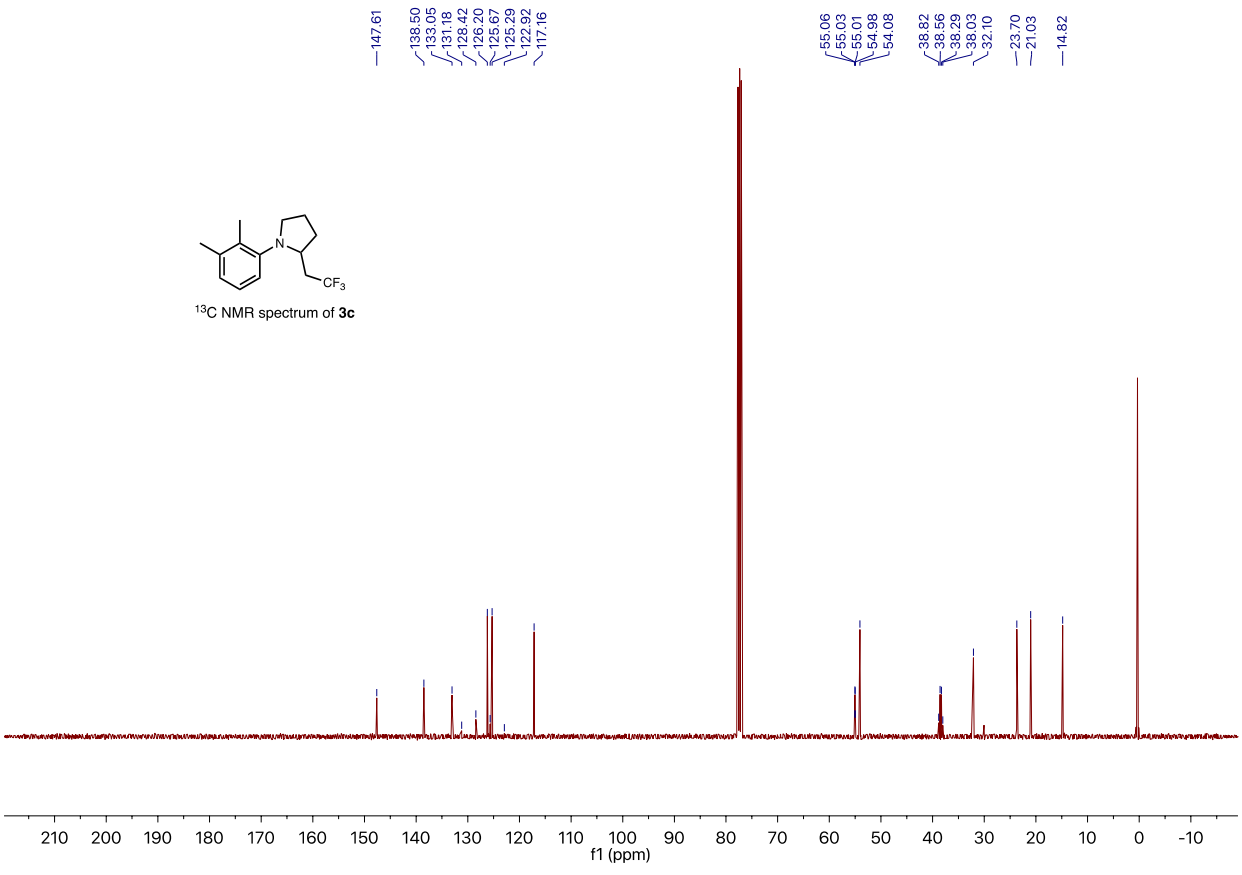
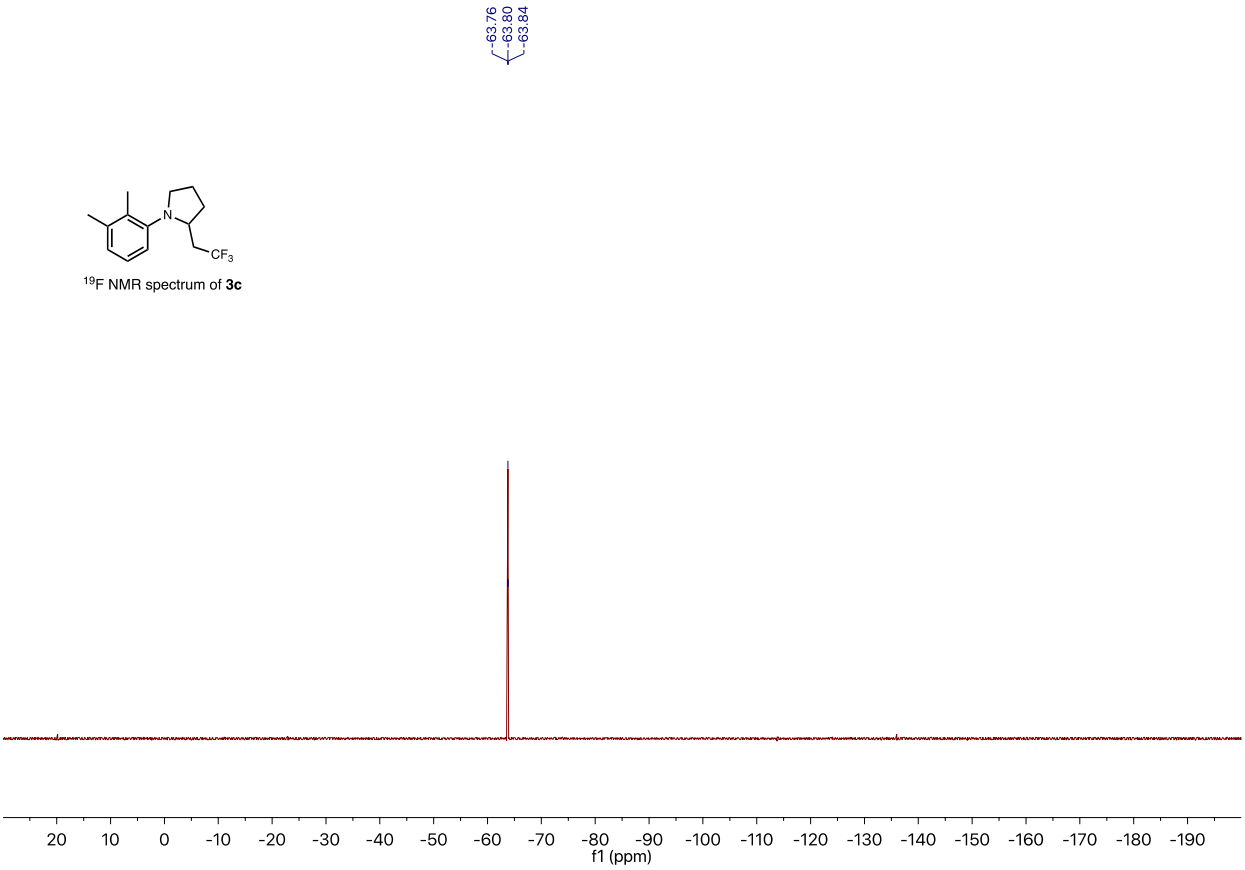
X. NMR Spectra



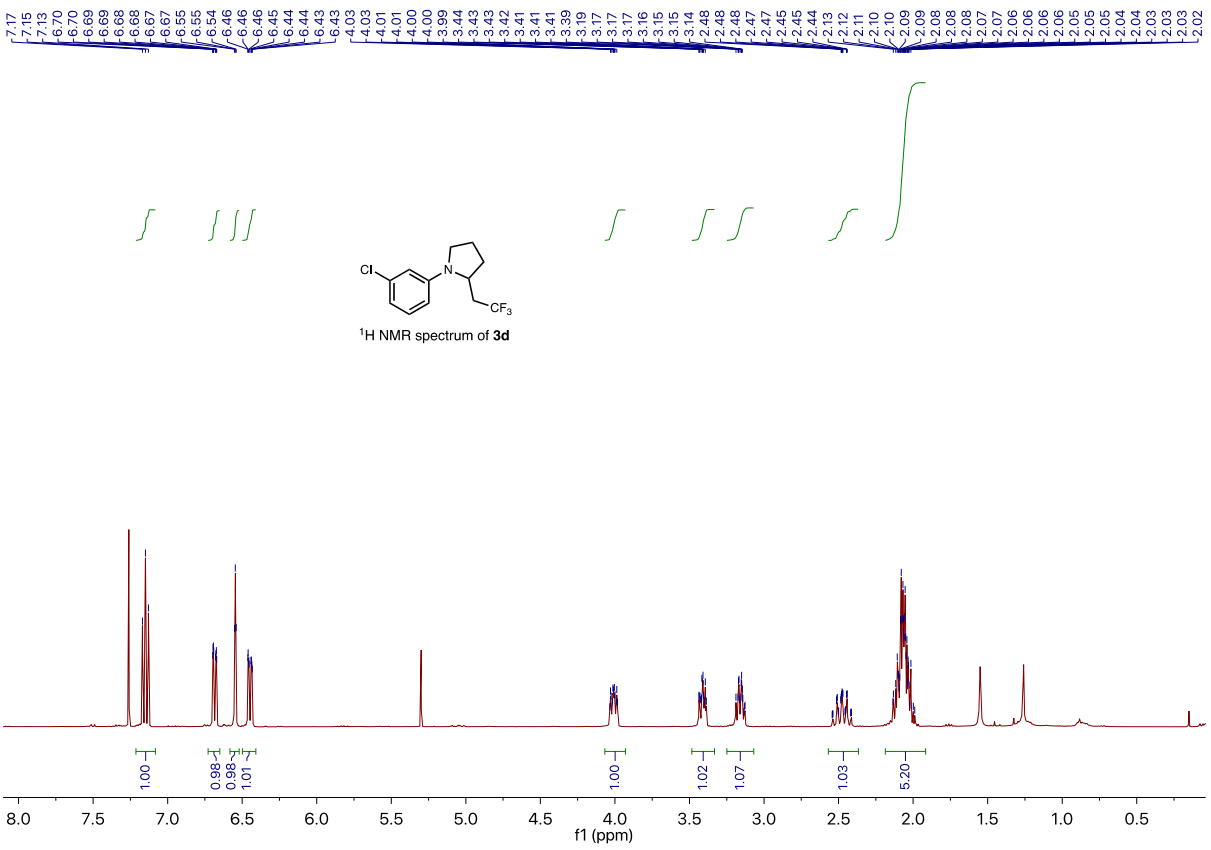
^{1}H NMR spectrum of **3c**



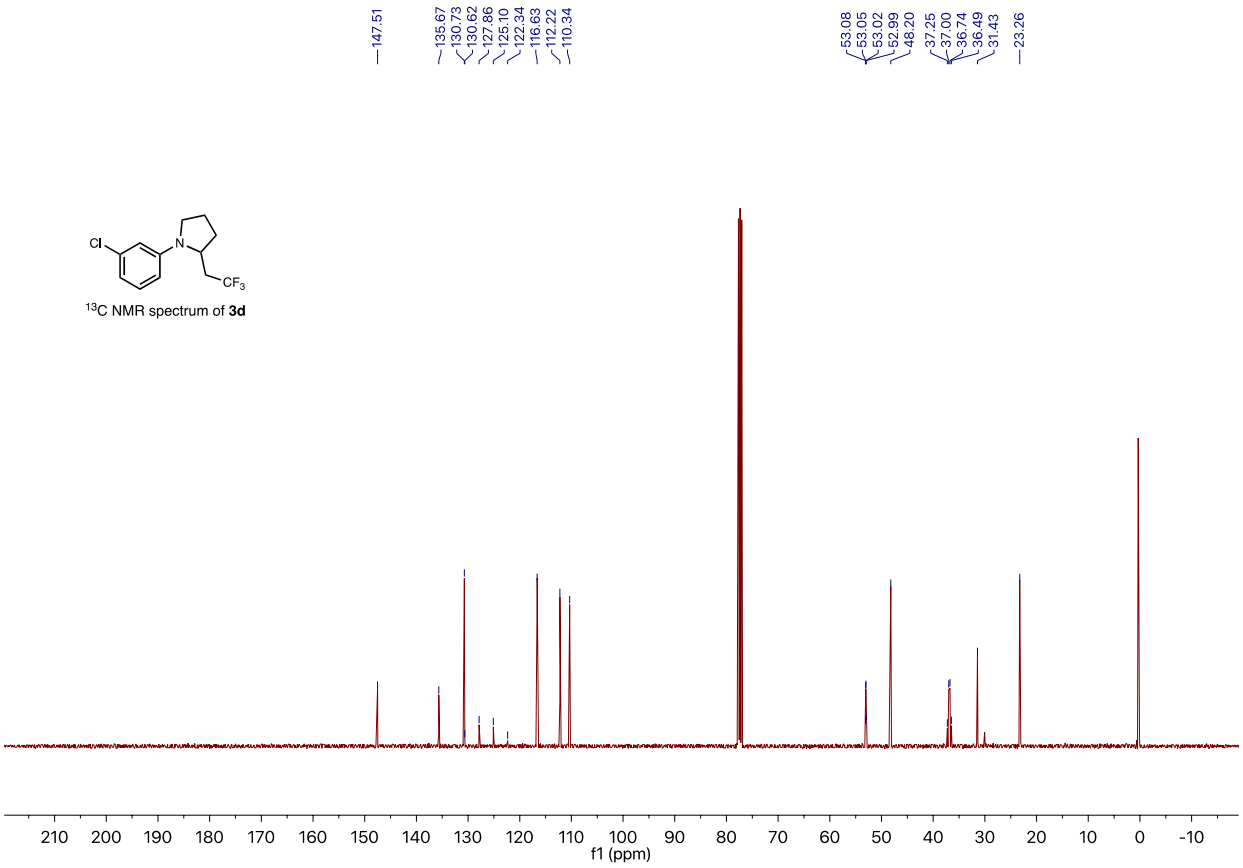
^{13}C NMR spectrum of **3c**



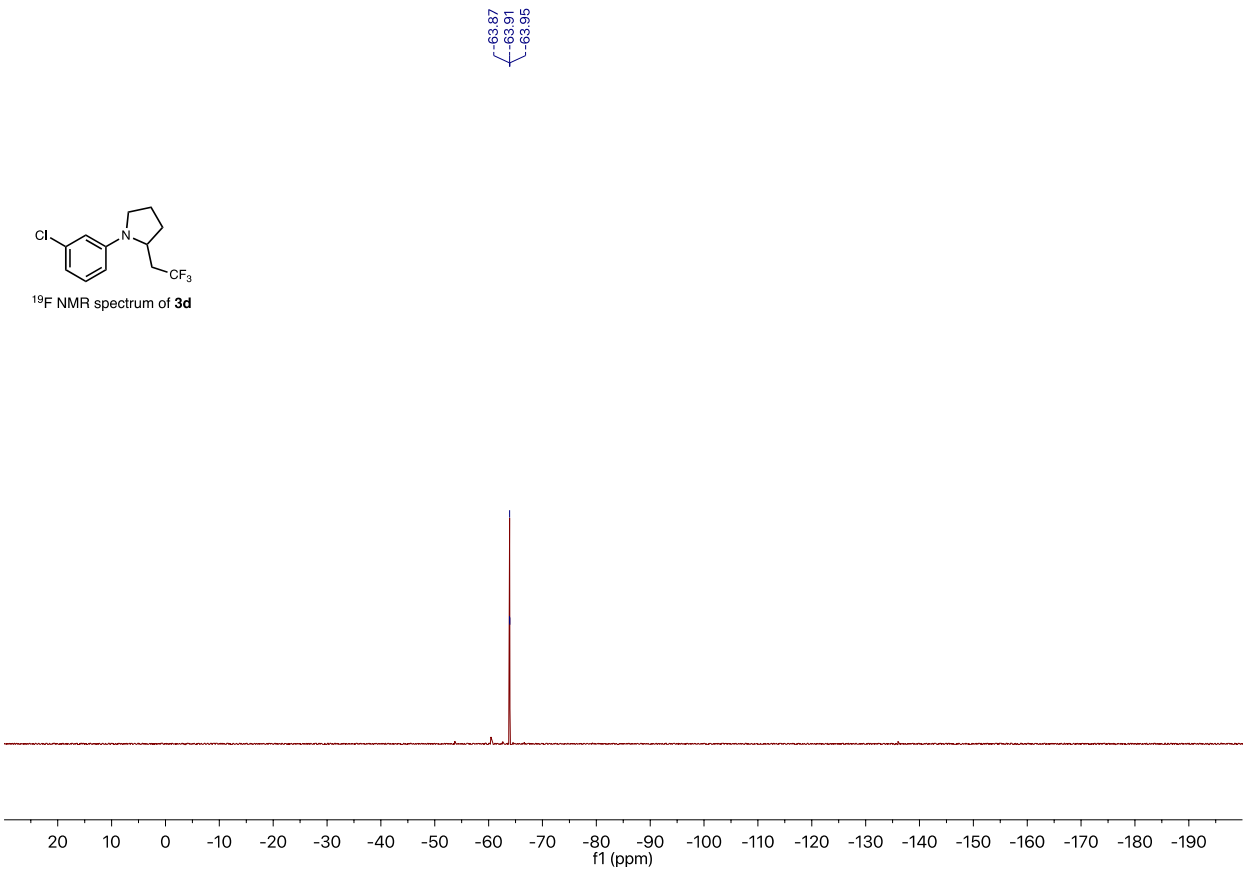
^{19}F NMR spectrum of **3c**



^{1}H NMR spectrum of **3d**



^{13}C NMR spectrum of **3d**



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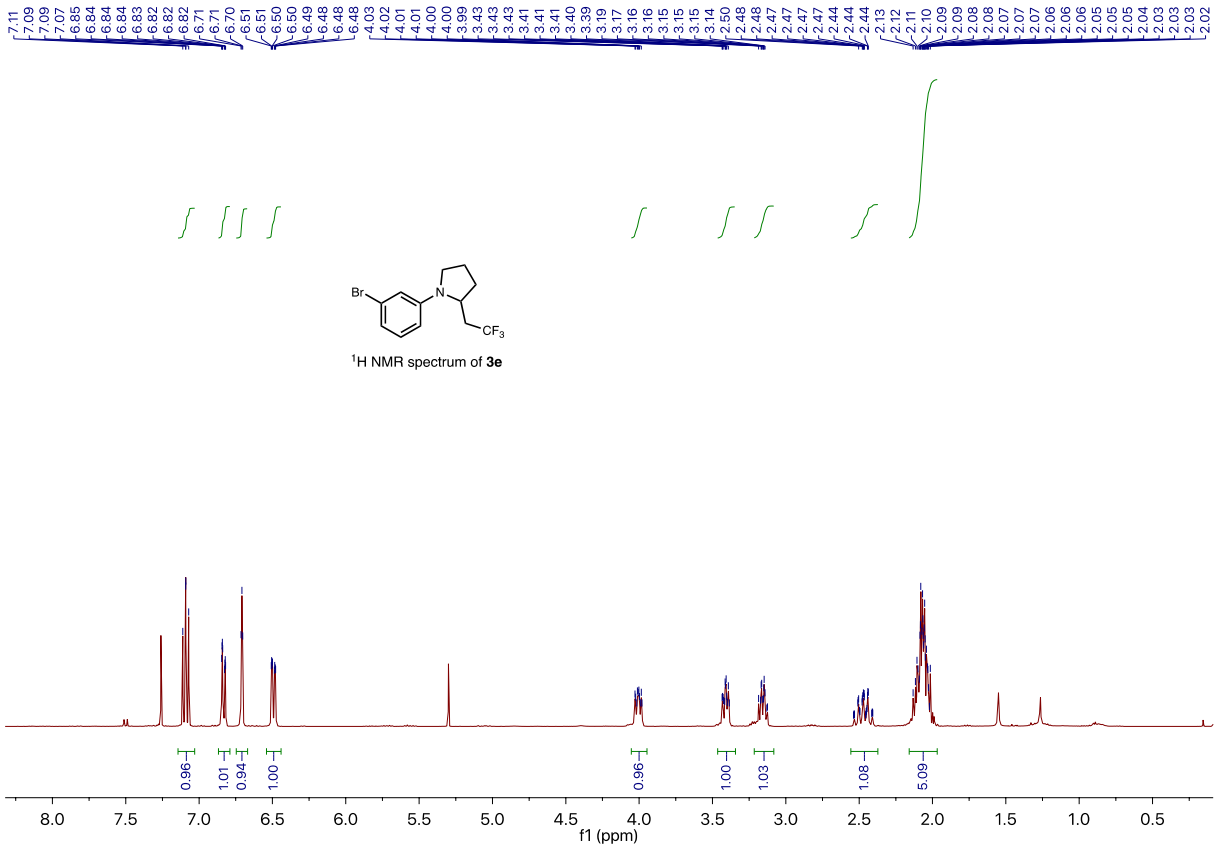
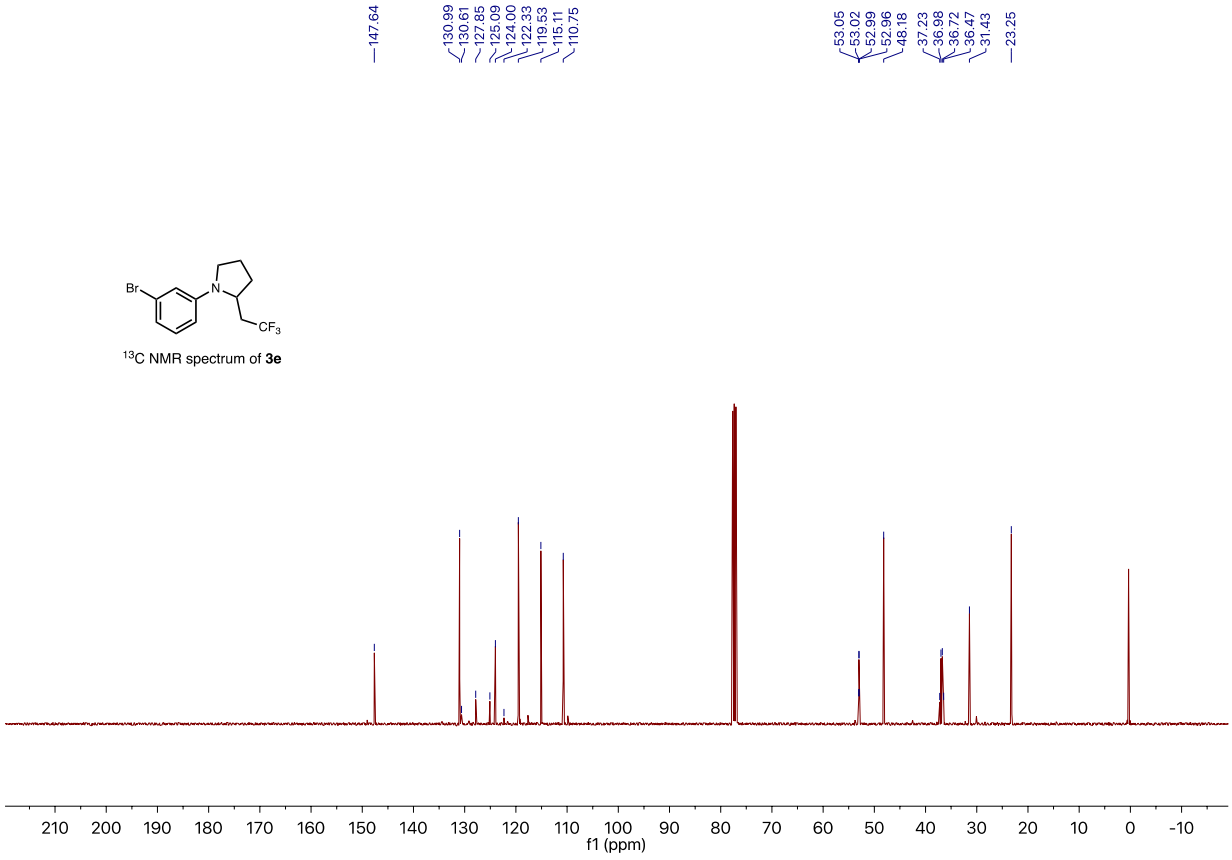
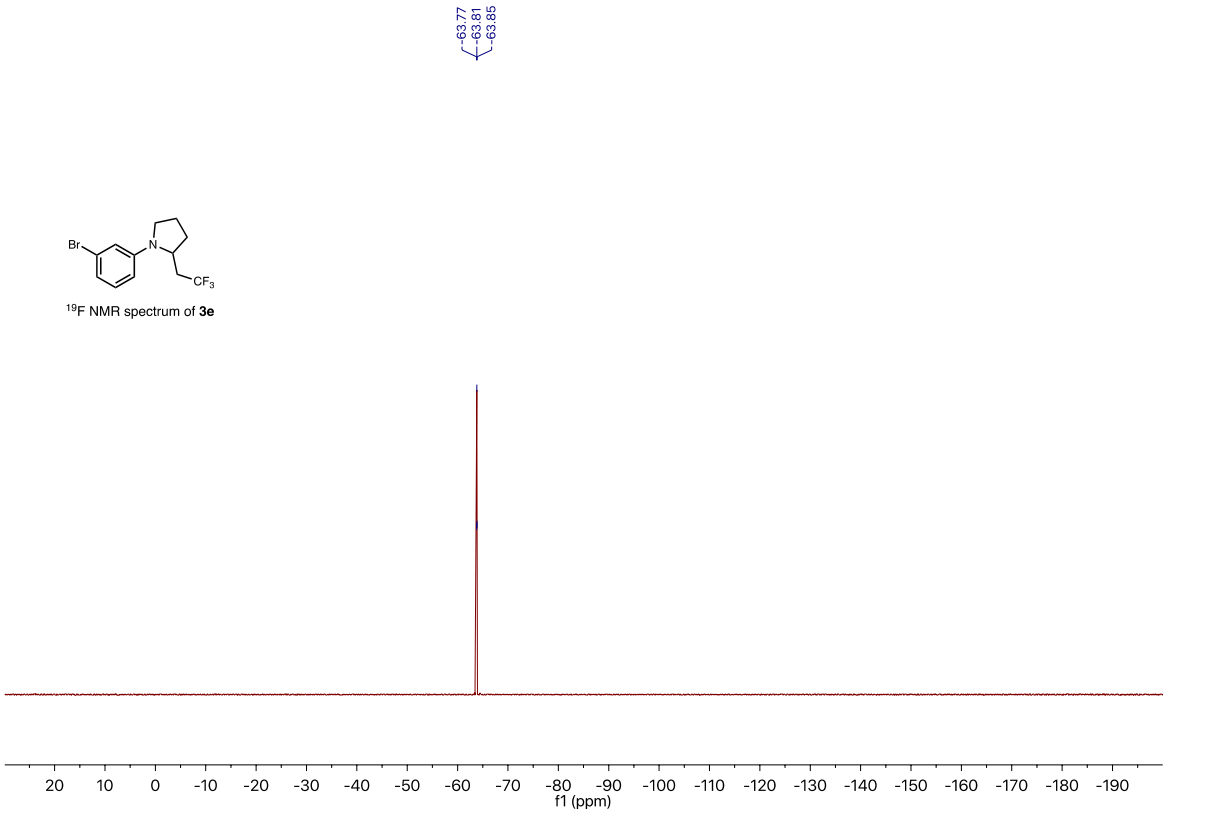


^{1}H NMR spectrum of **3e**



^{13}C NMR spectrum of **3e**



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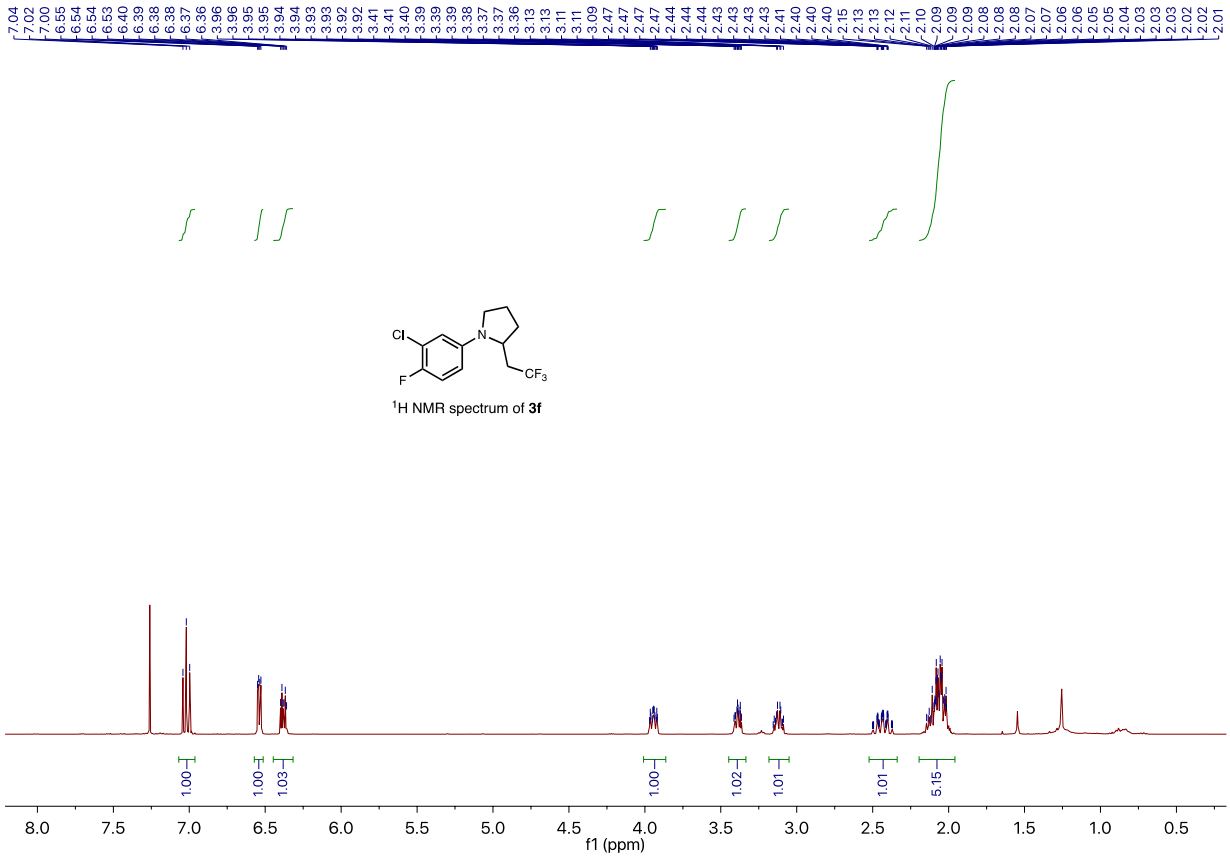
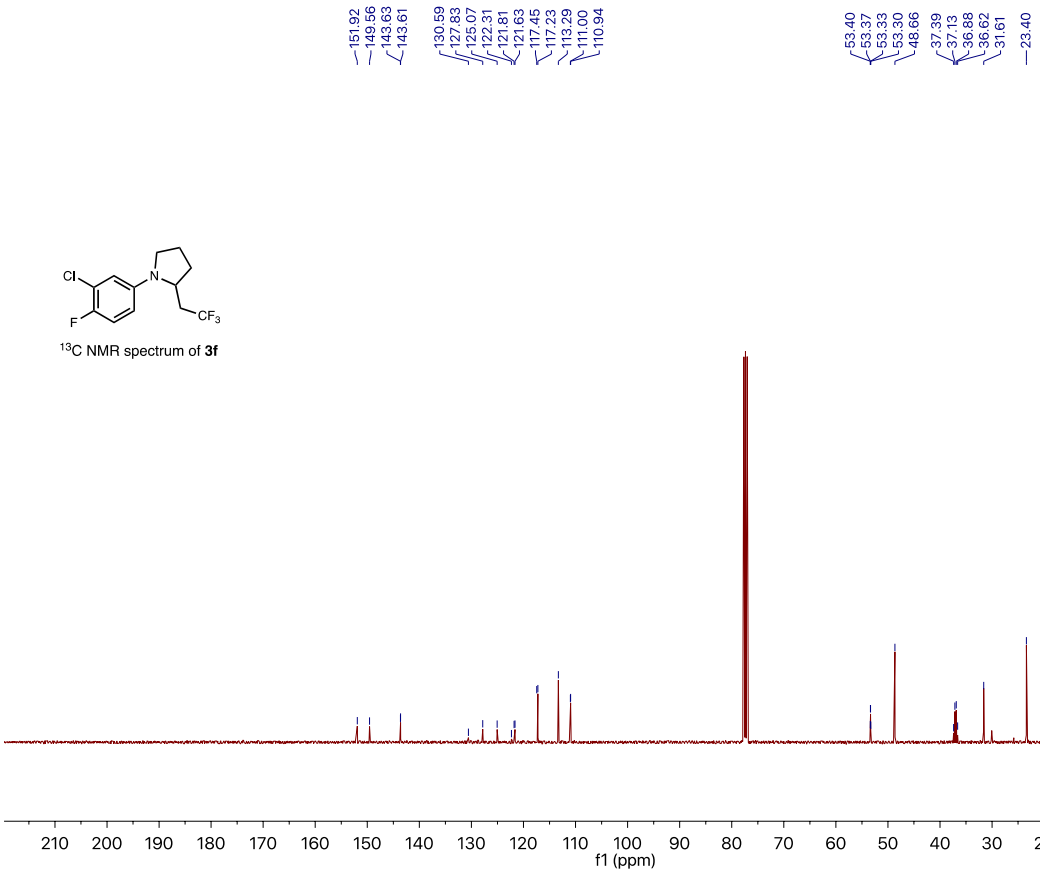
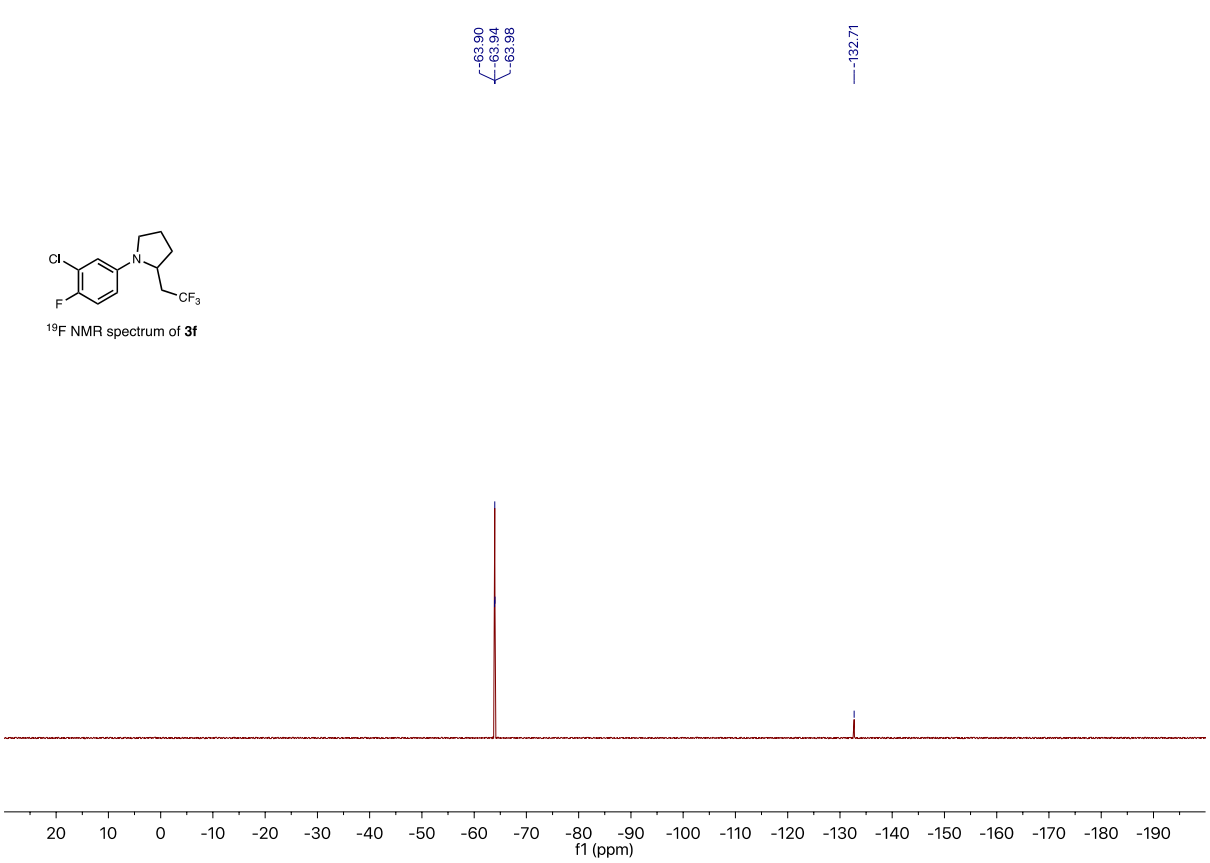


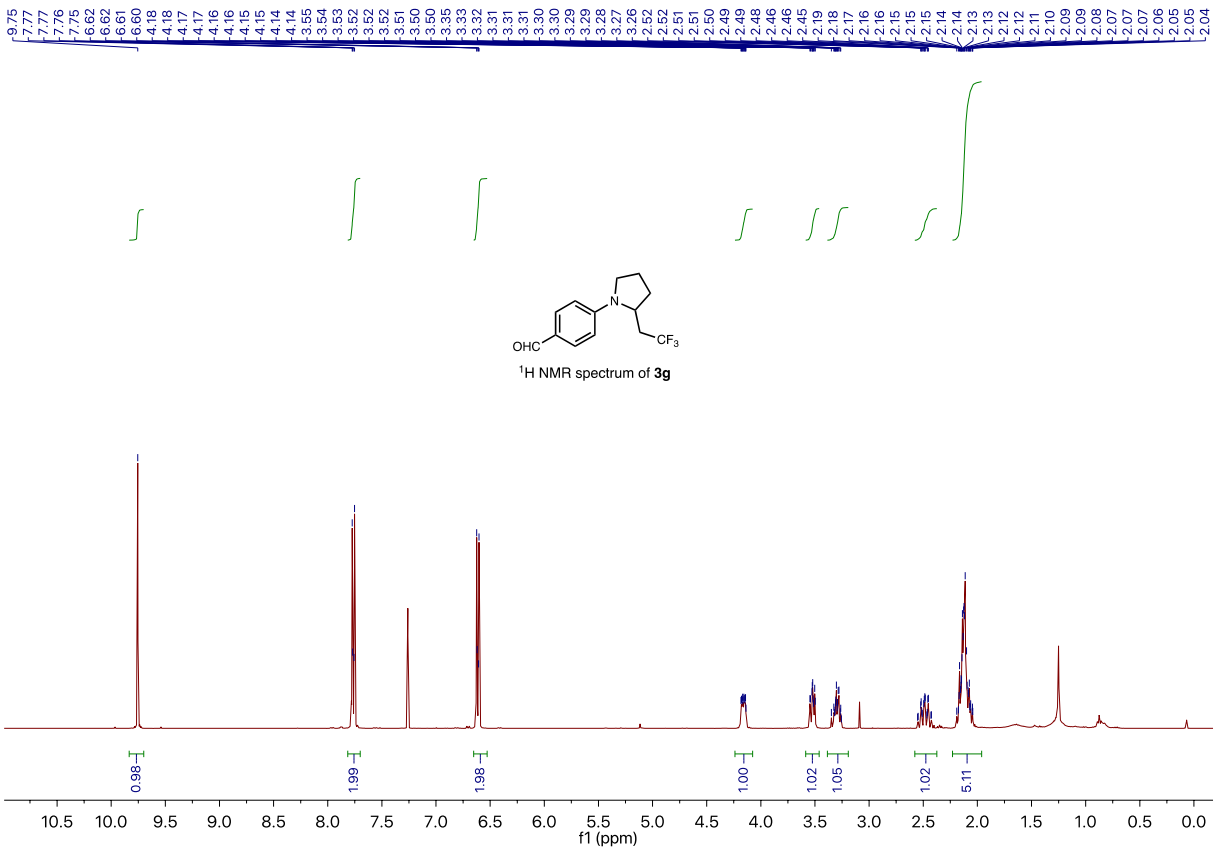
^{1}H NMR spectrum of **3f**



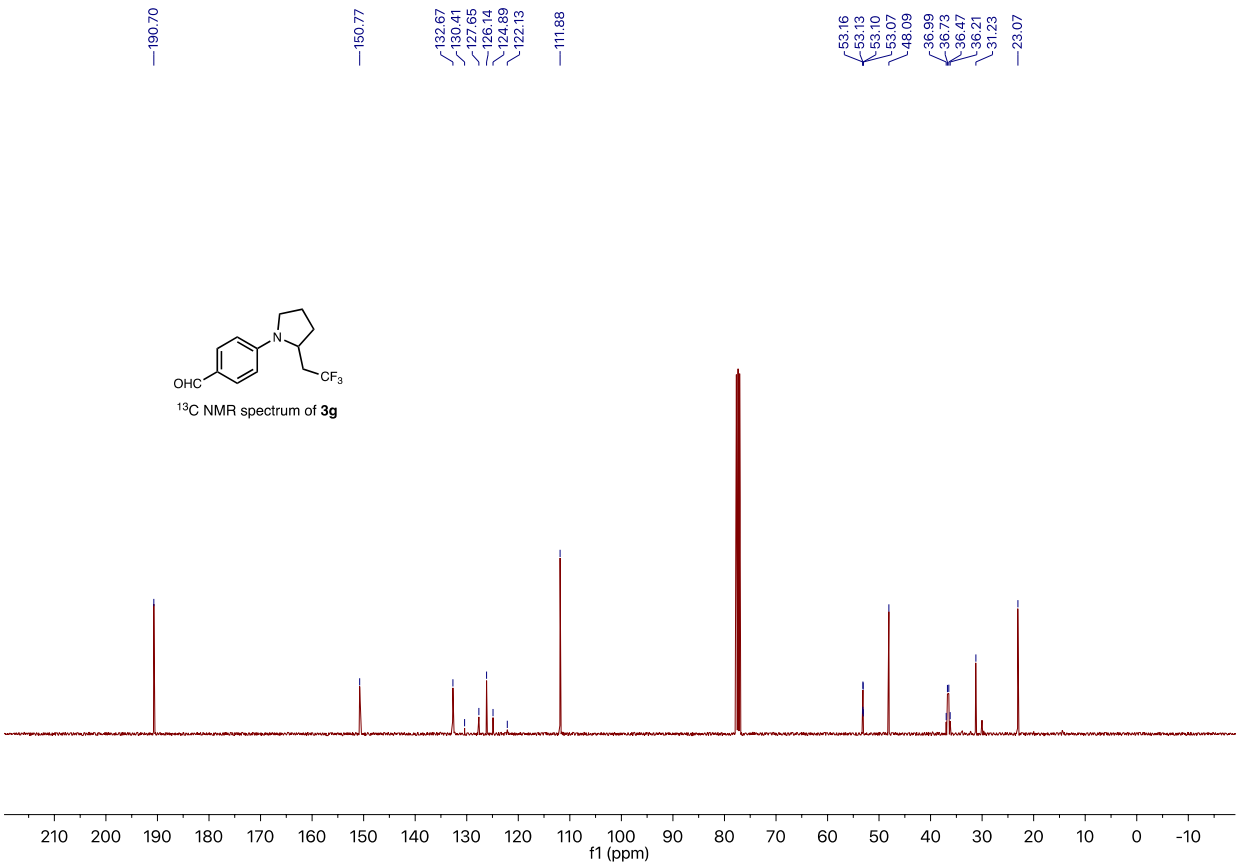
^{13}C NMR spectrum of **3f**



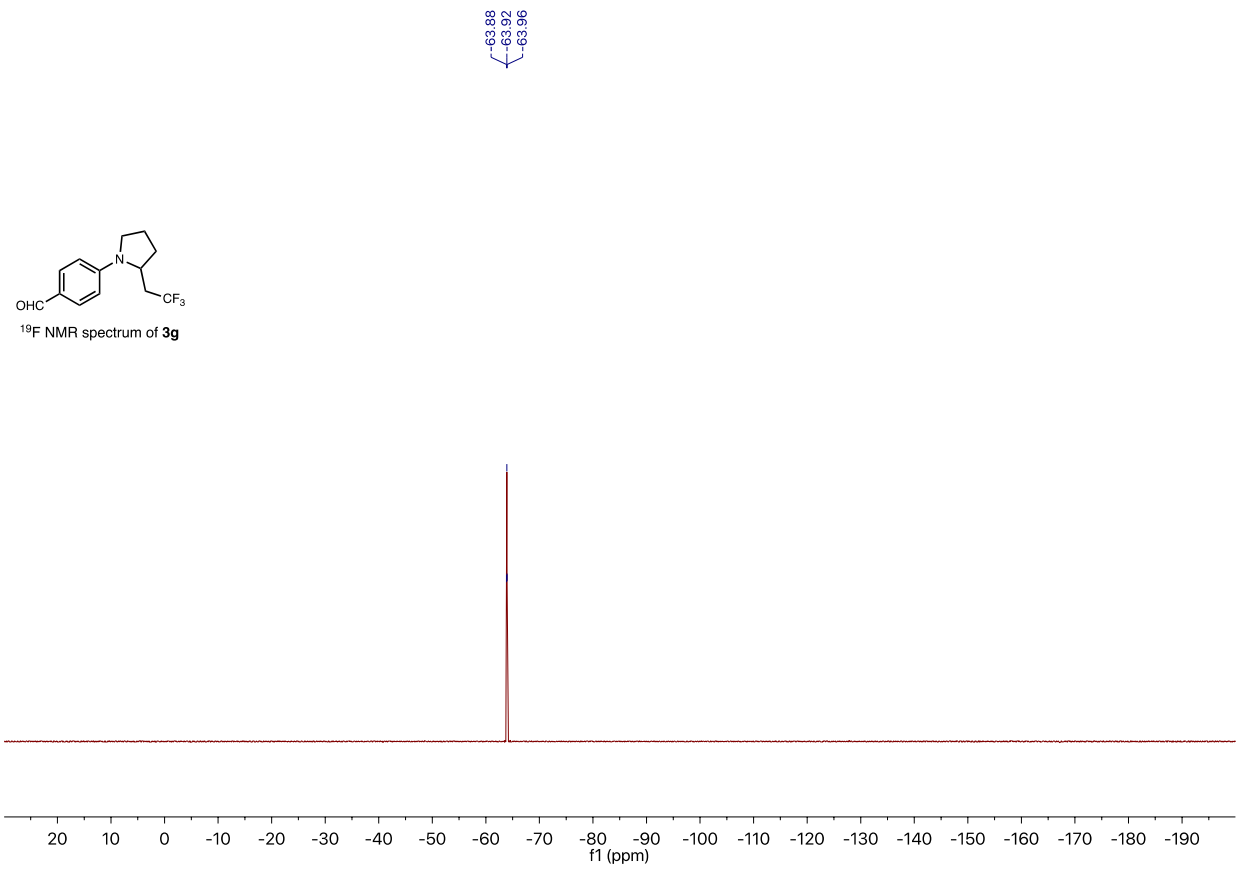
^{19}F NMR spectrum of **3f**



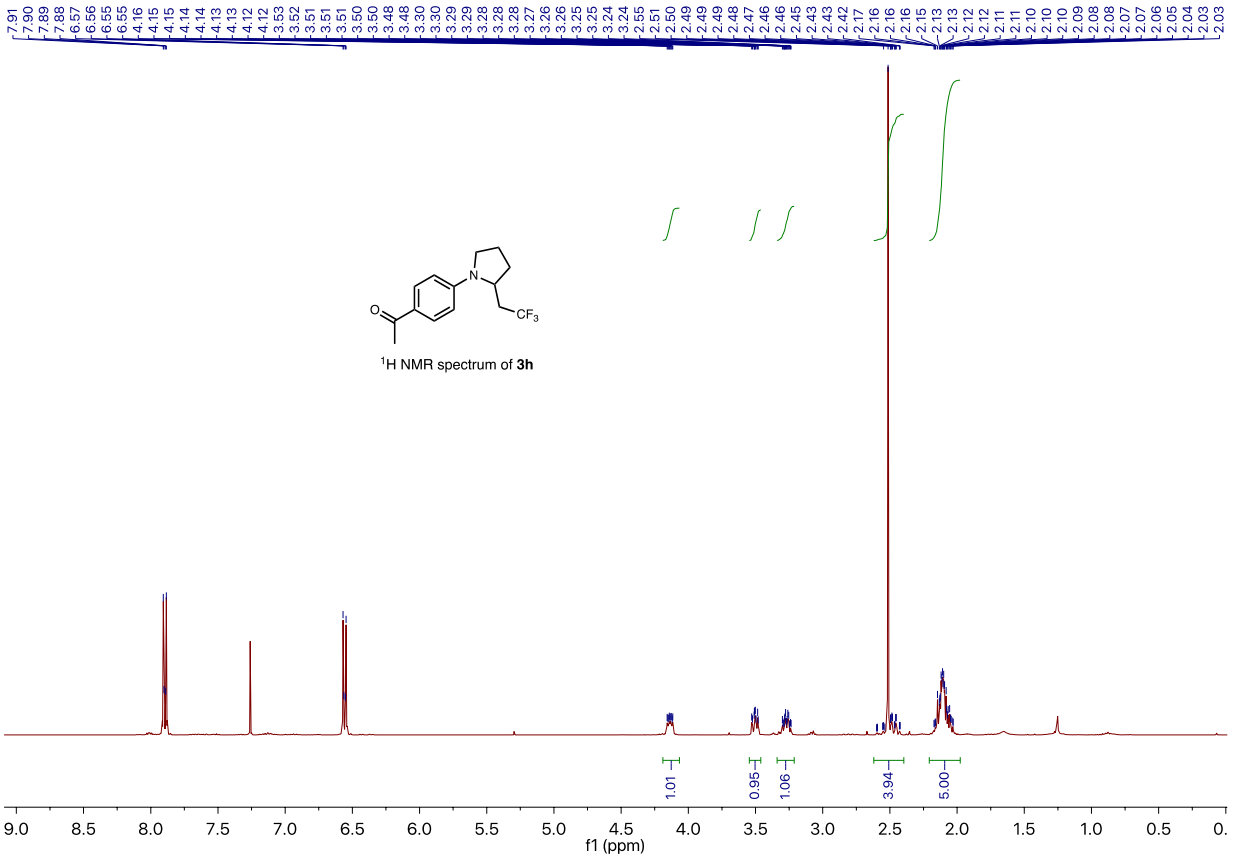
^{1}H NMR spectrum of **3g**



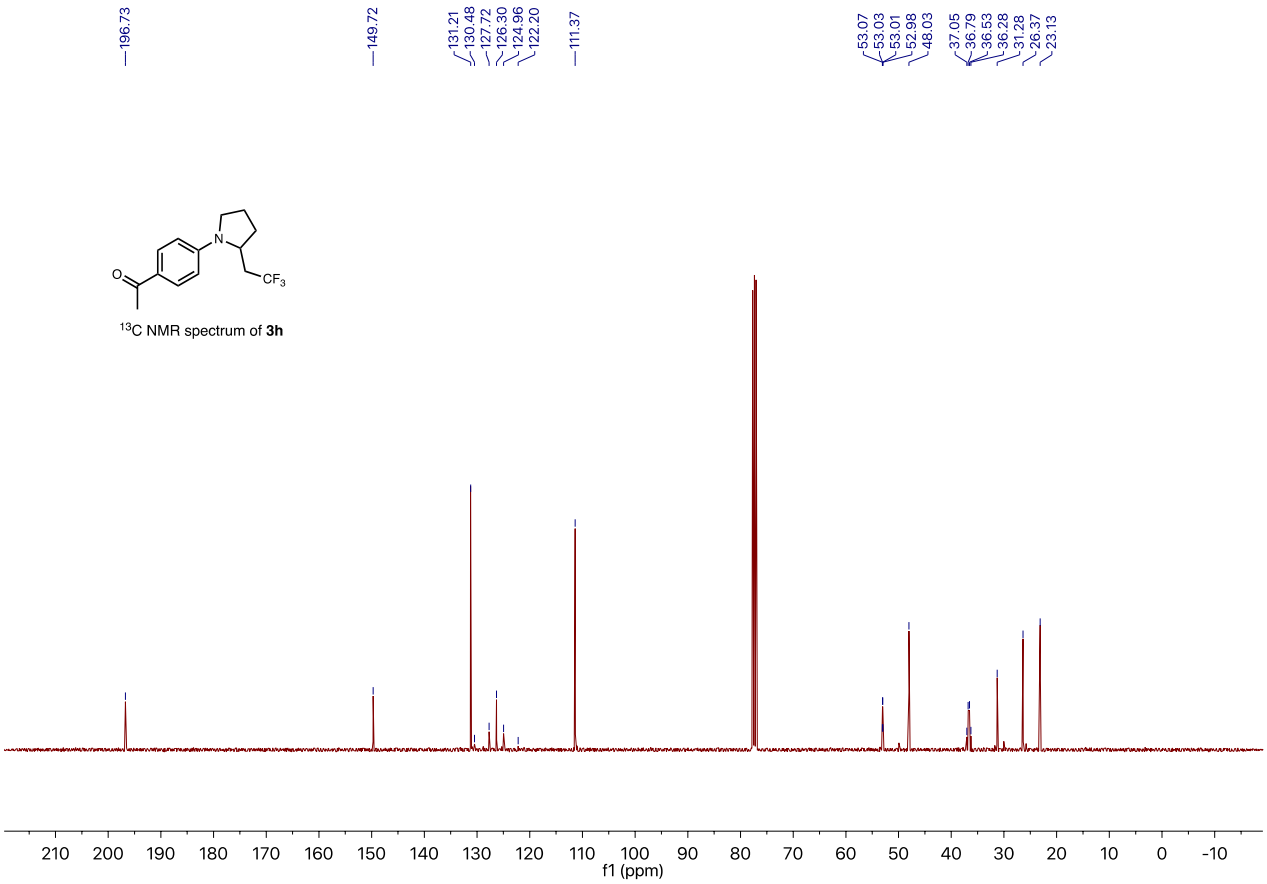
^{13}C NMR spectrum of **3g**



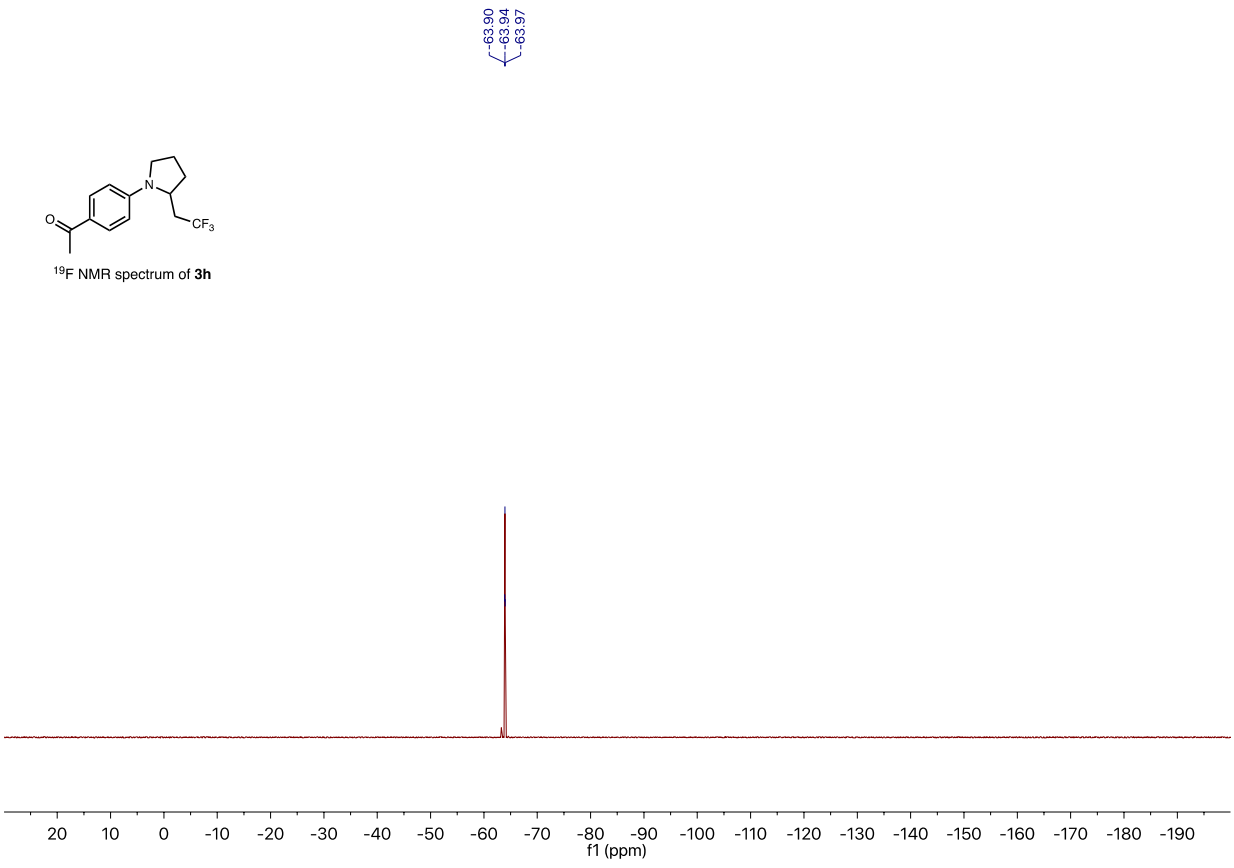
^{19}F NMR spectrum of **3g**



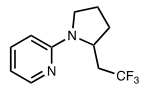
^{1}H NMR spectrum of **3h**



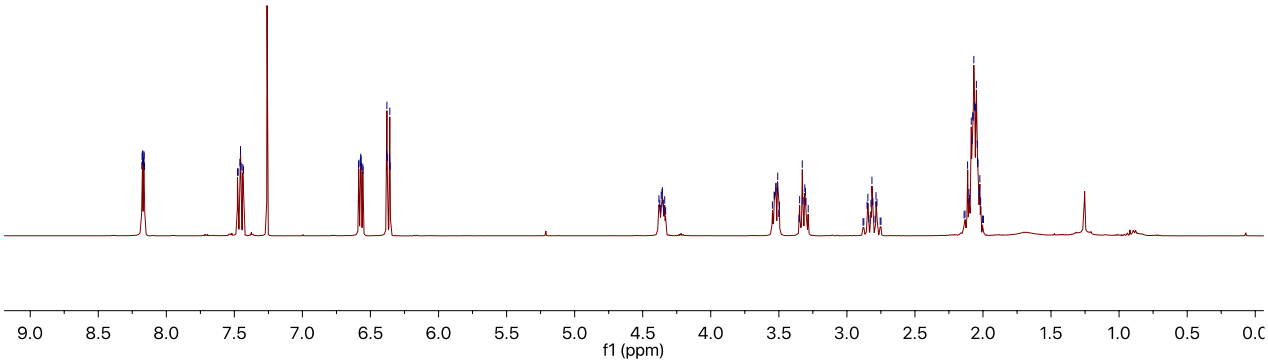
^{13}C NMR spectrum of **3h**

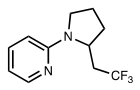


^{19}F NMR spectrum of **3h**

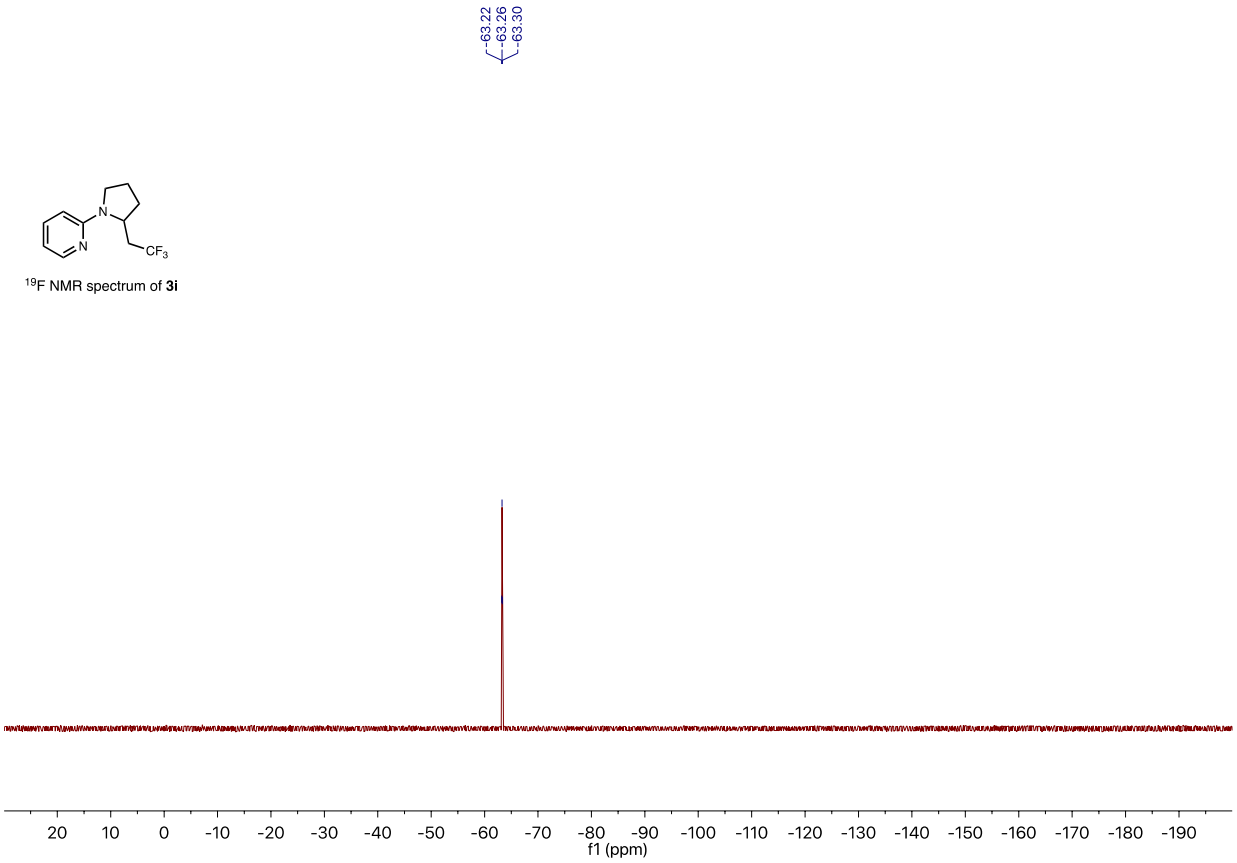


^{1}H NMR spectrum of **3i**

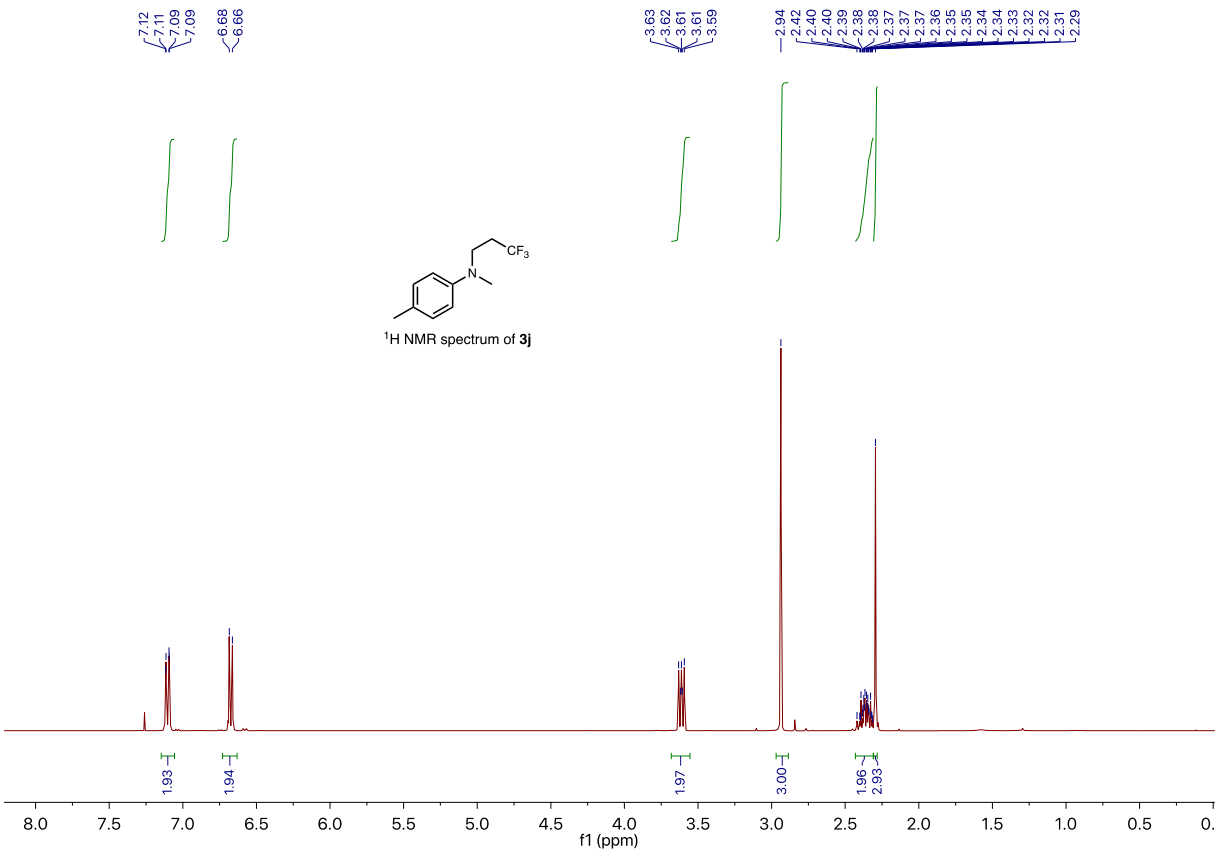




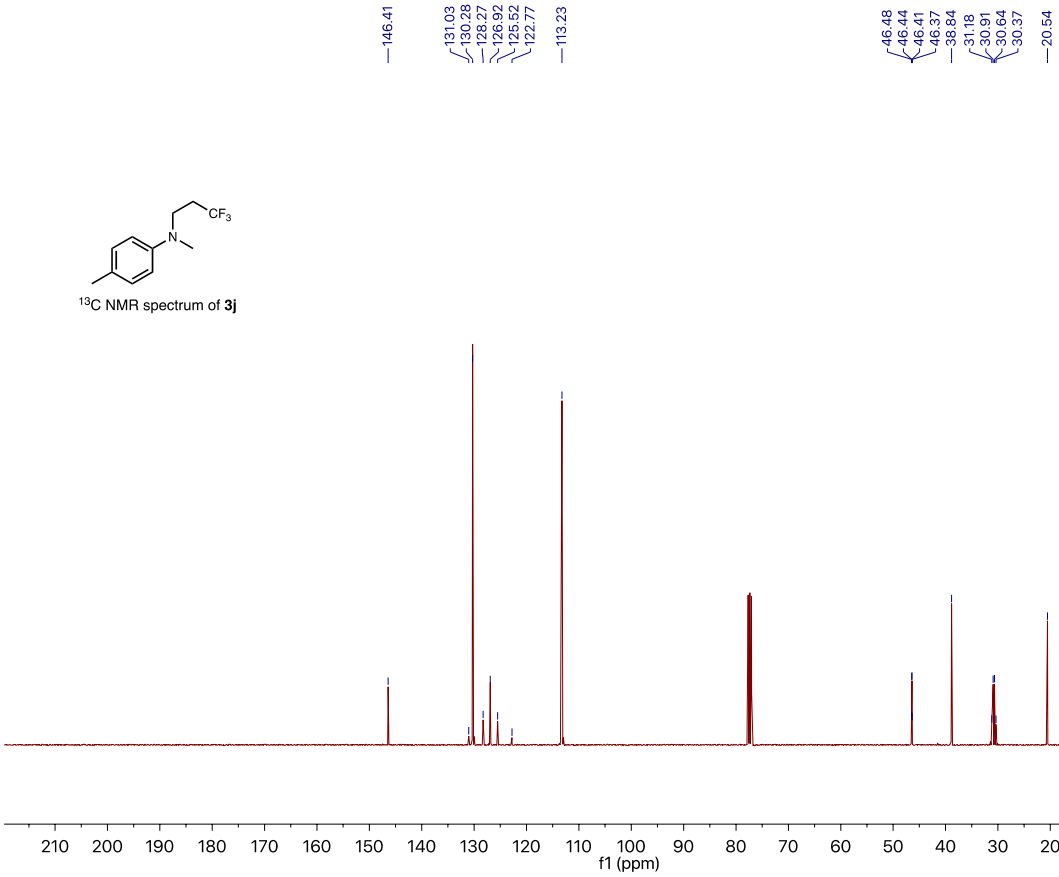
^{13}C NMR spectrum of **3i**



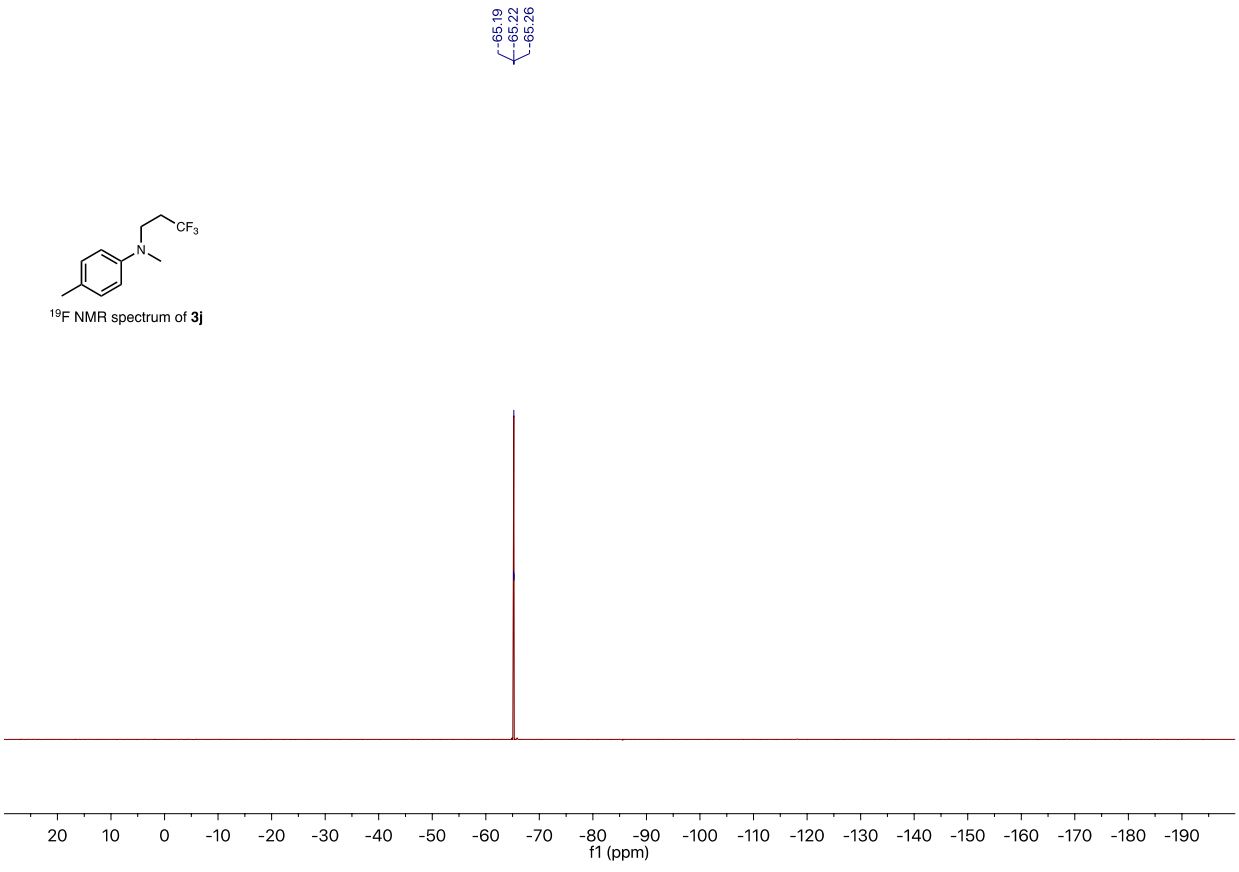
^{19}F NMR spectrum of **3i**



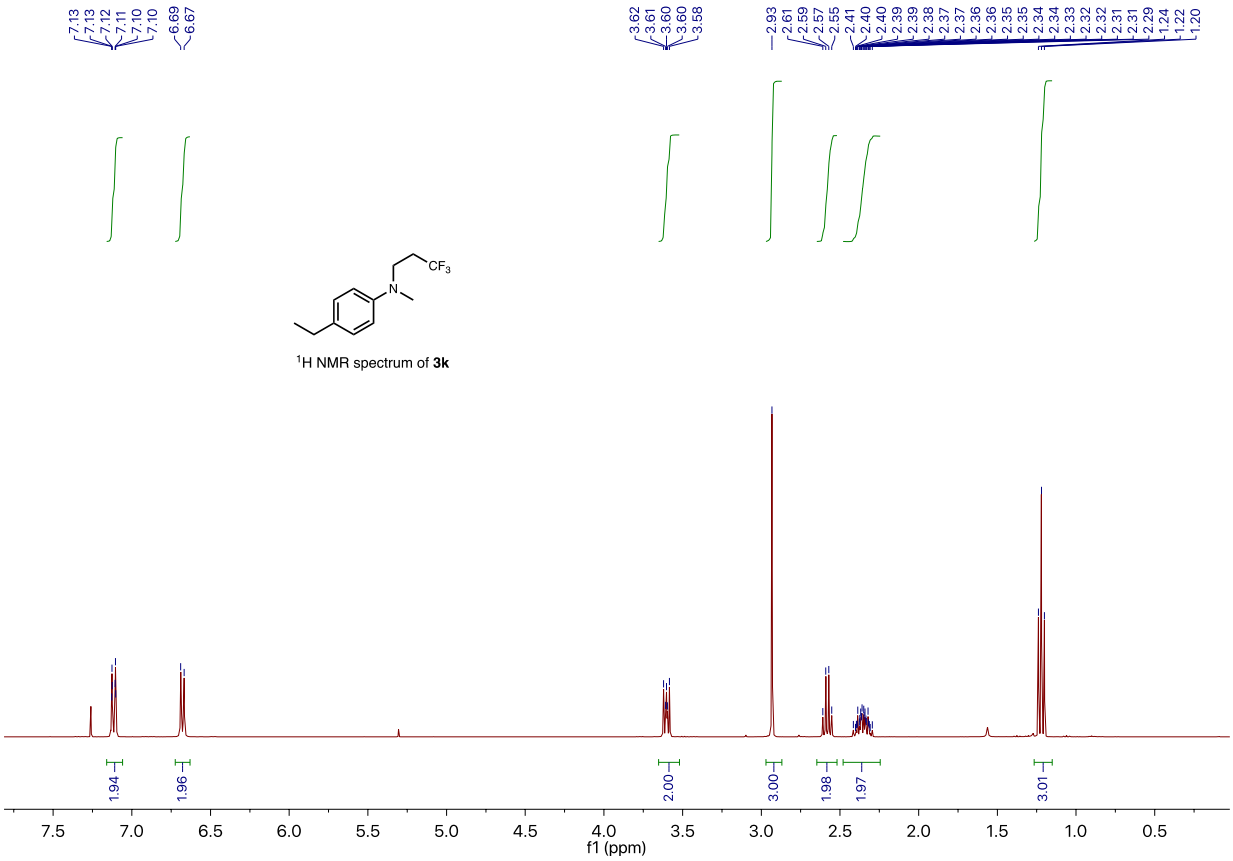
^{1}H NMR spectrum of **3j**



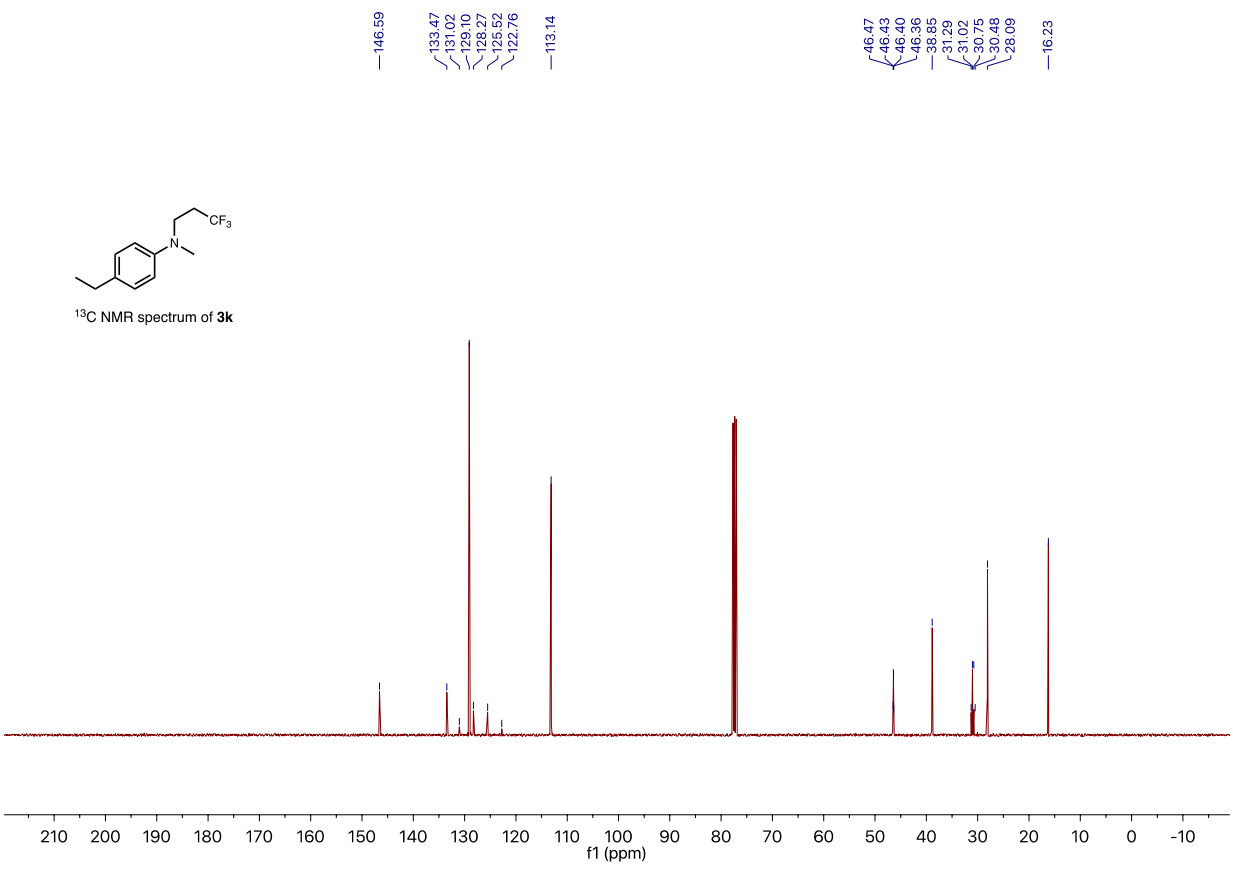
^{13}C NMR spectrum of **3j**



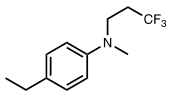
^{19}F NMR spectrum of **3j**



^{1}H NMR spectrum of **3k**

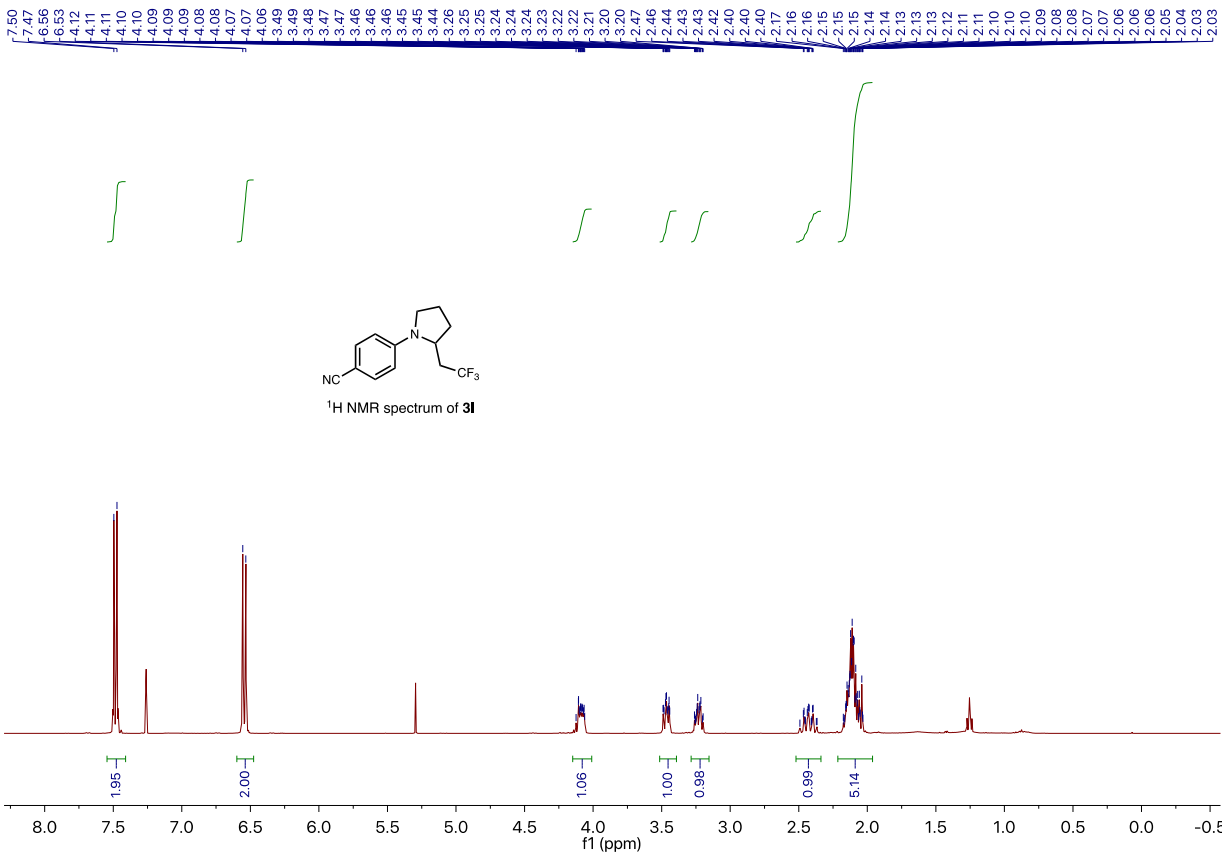


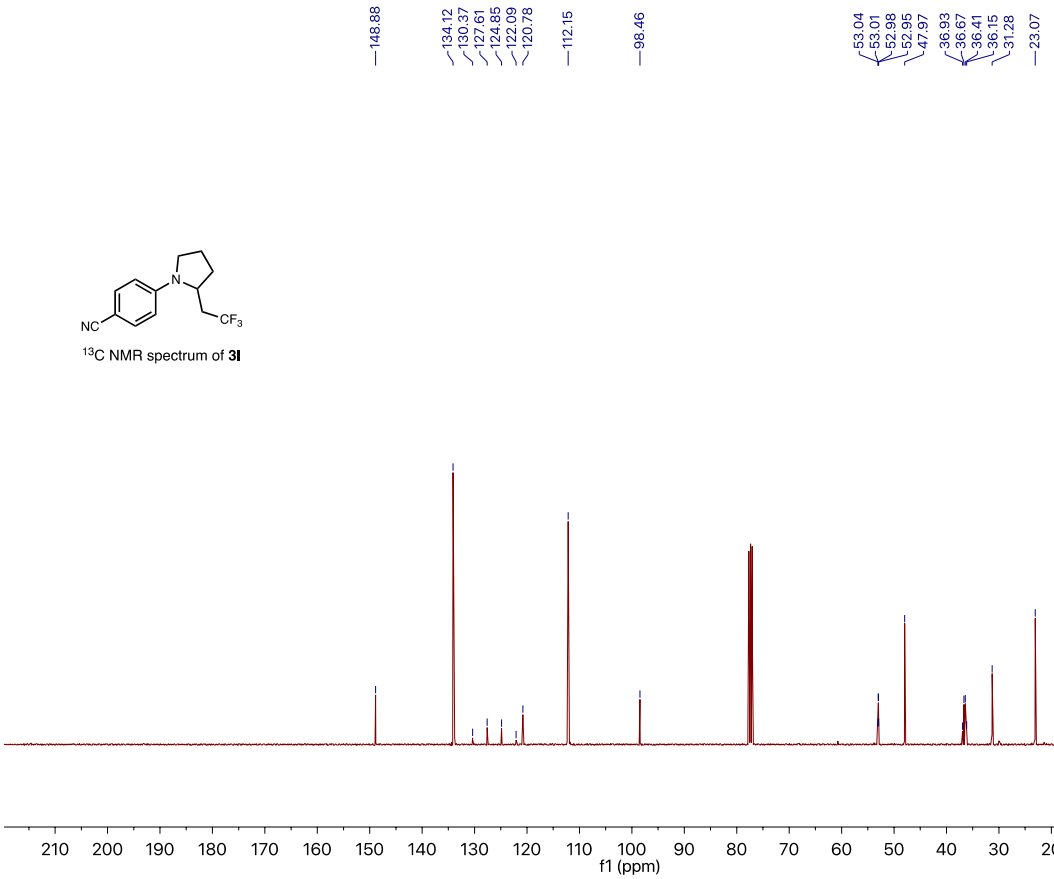
^{13}C NMR spectrum of **3k**



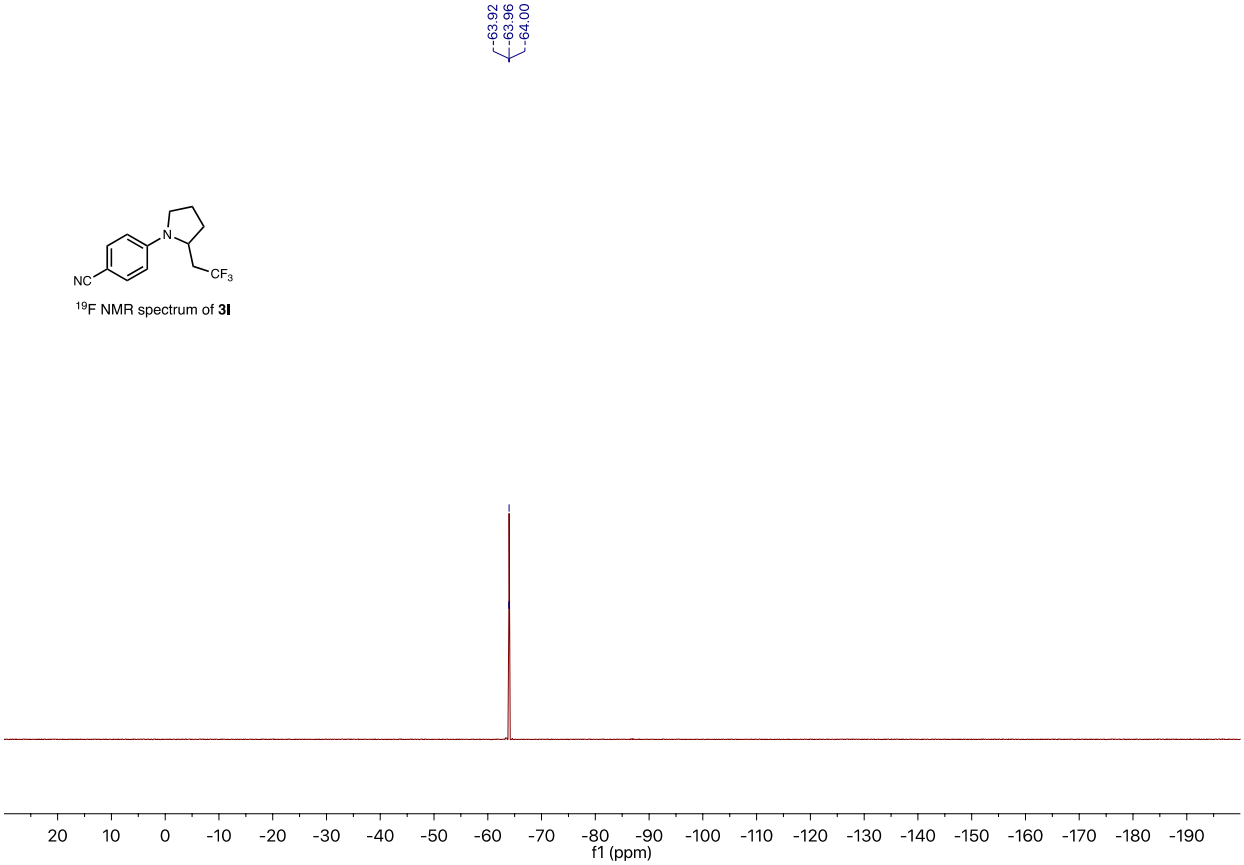
^{19}F NMR spectrum of **3k**

^{1}H NMR spectrum of **3l**

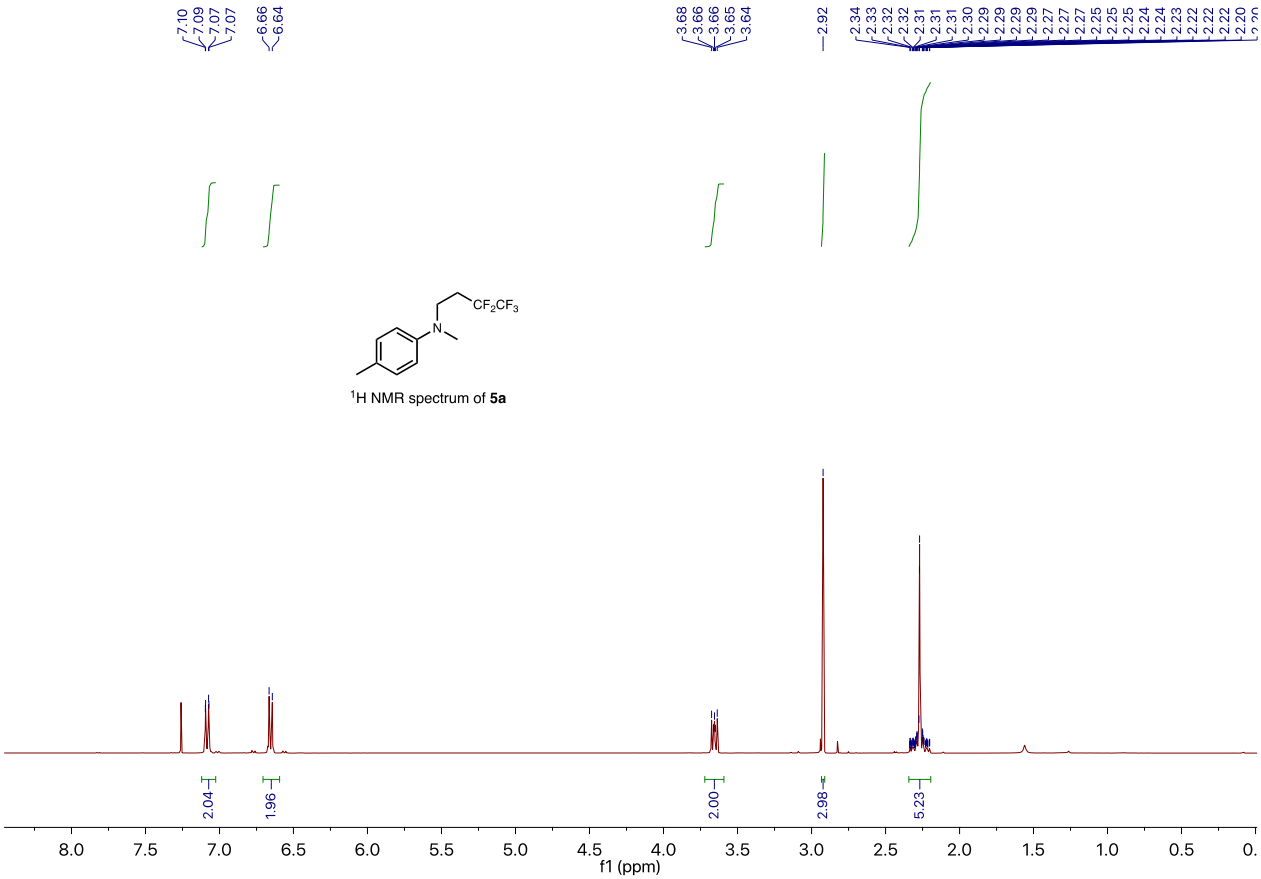




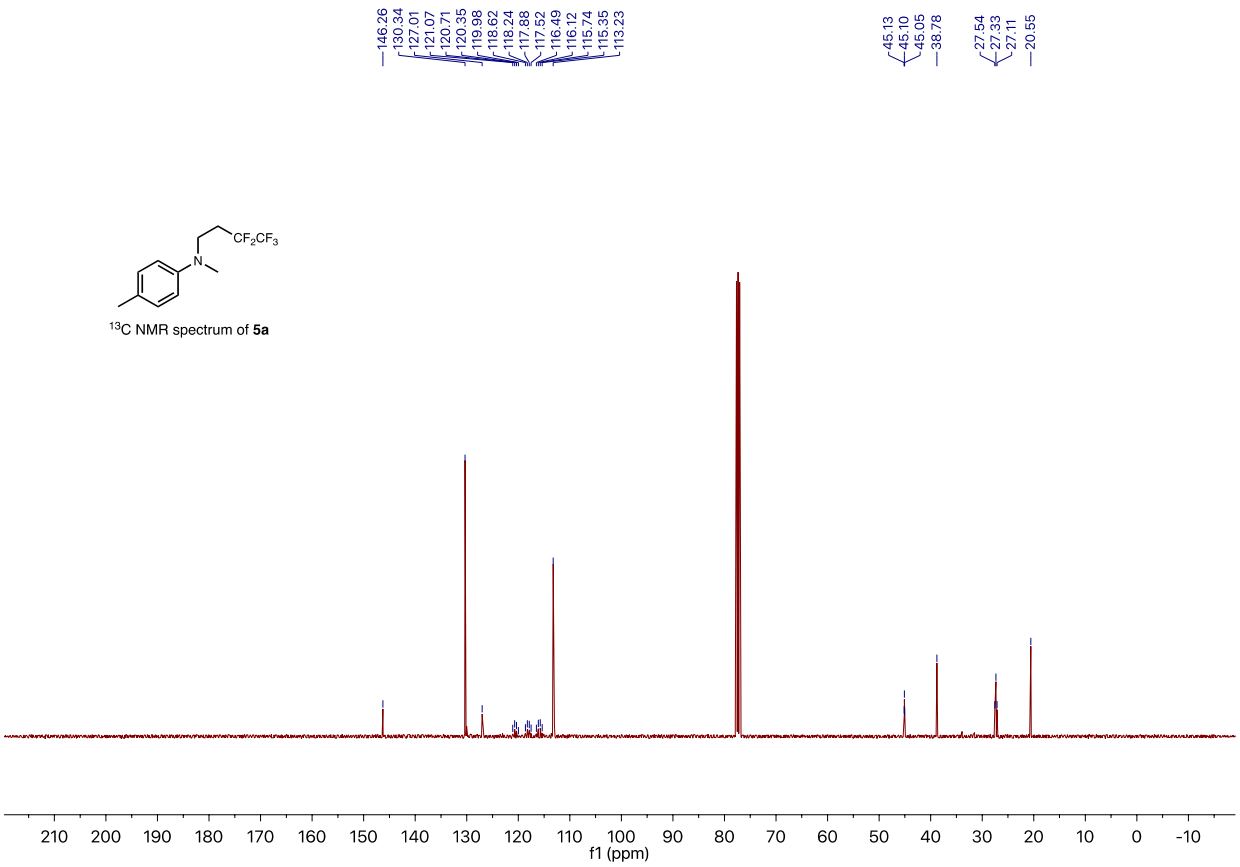
^{13}C NMR spectrum of **3l**



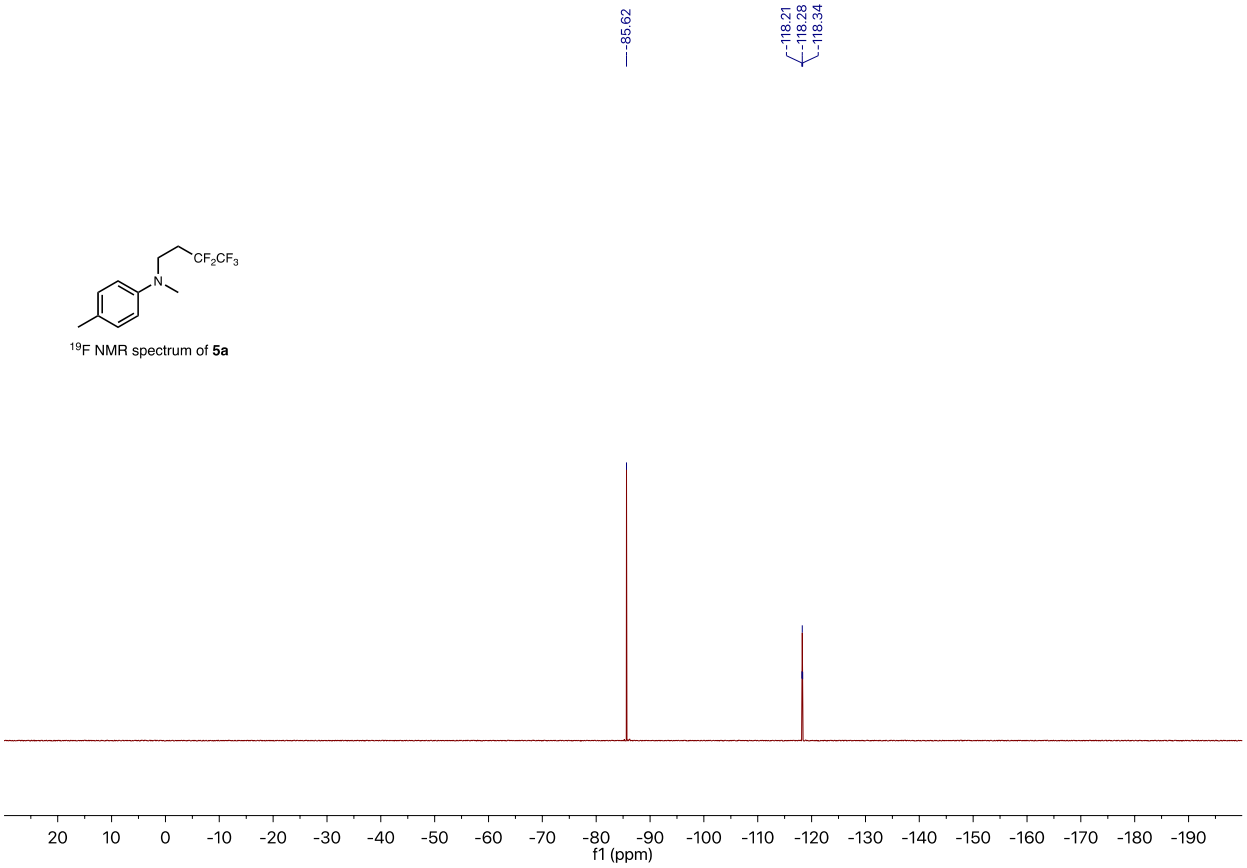
^{19}F NMR spectrum of **3l**



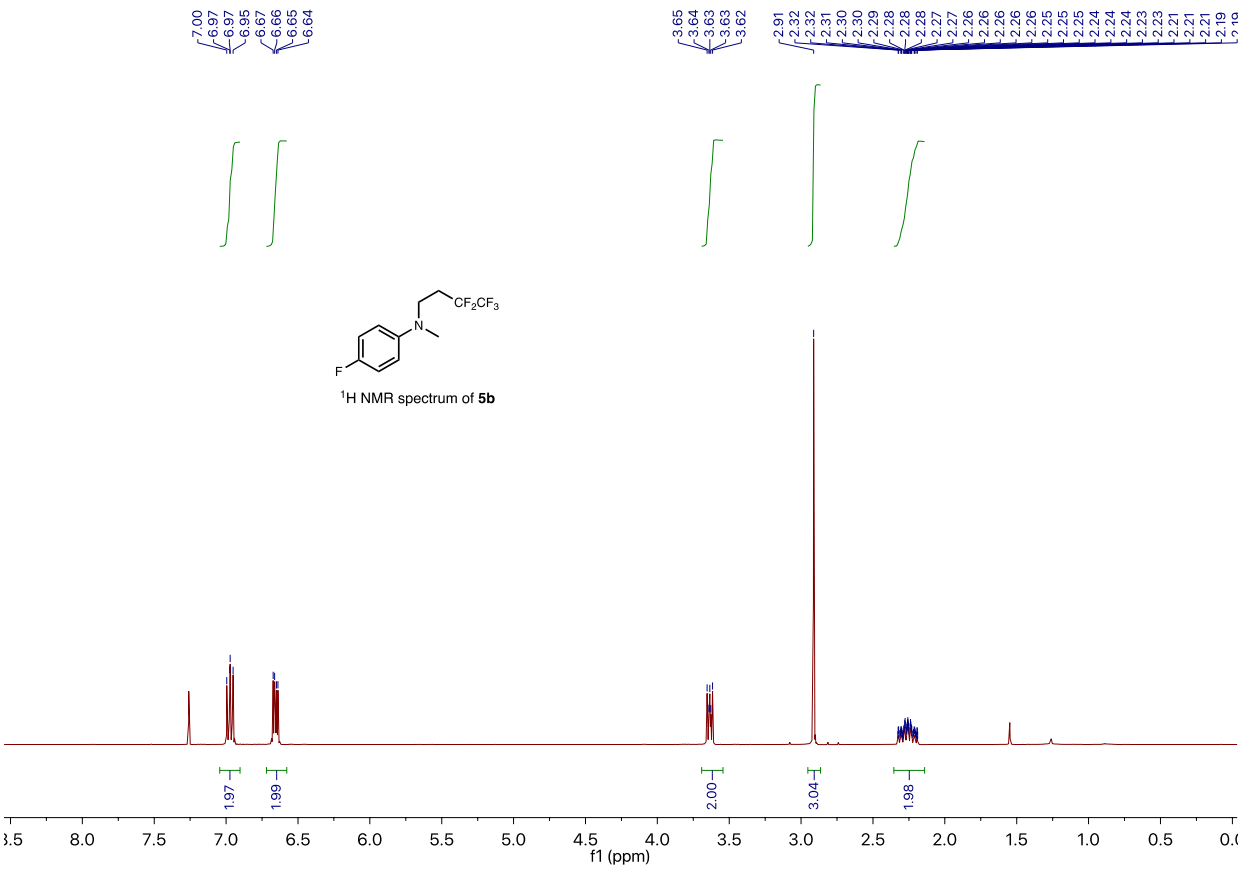
^{1}H NMR spectrum of **5a**



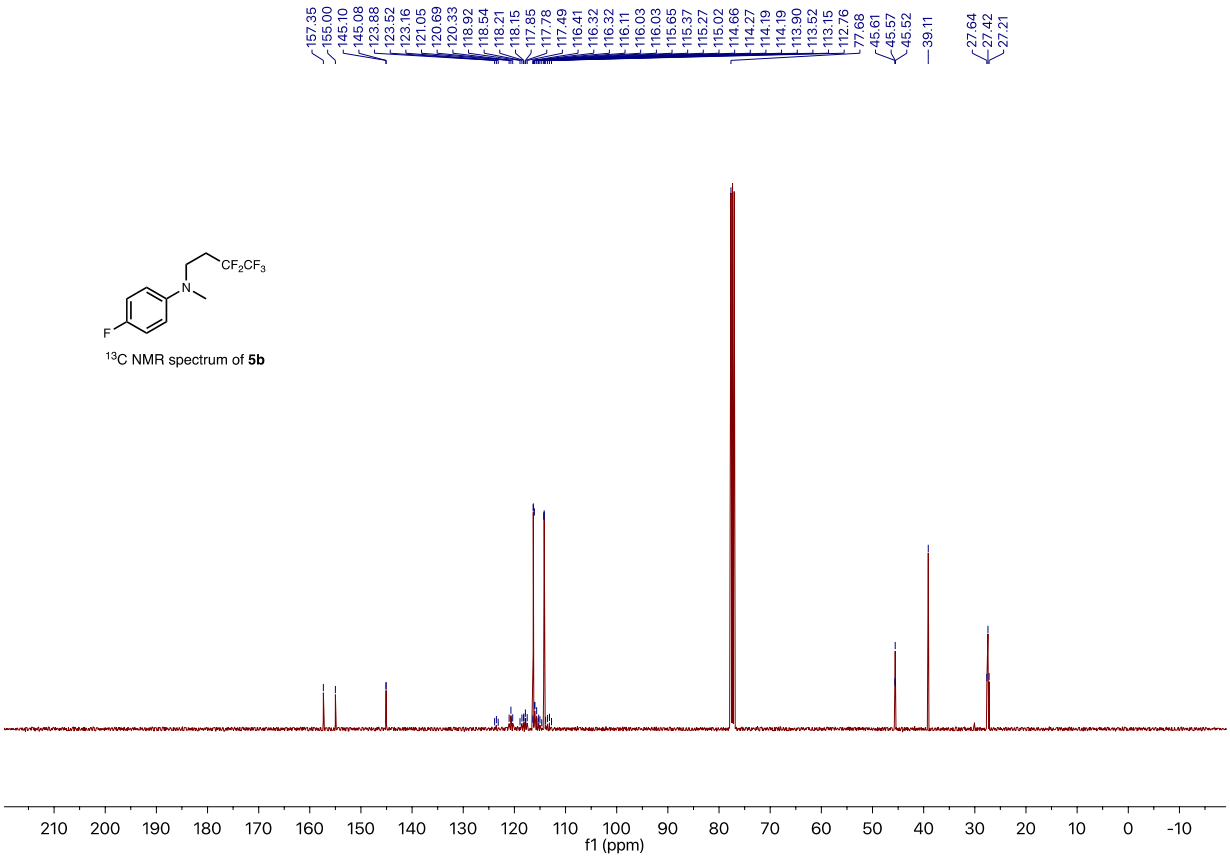
^{13}C NMR spectrum of **5a**



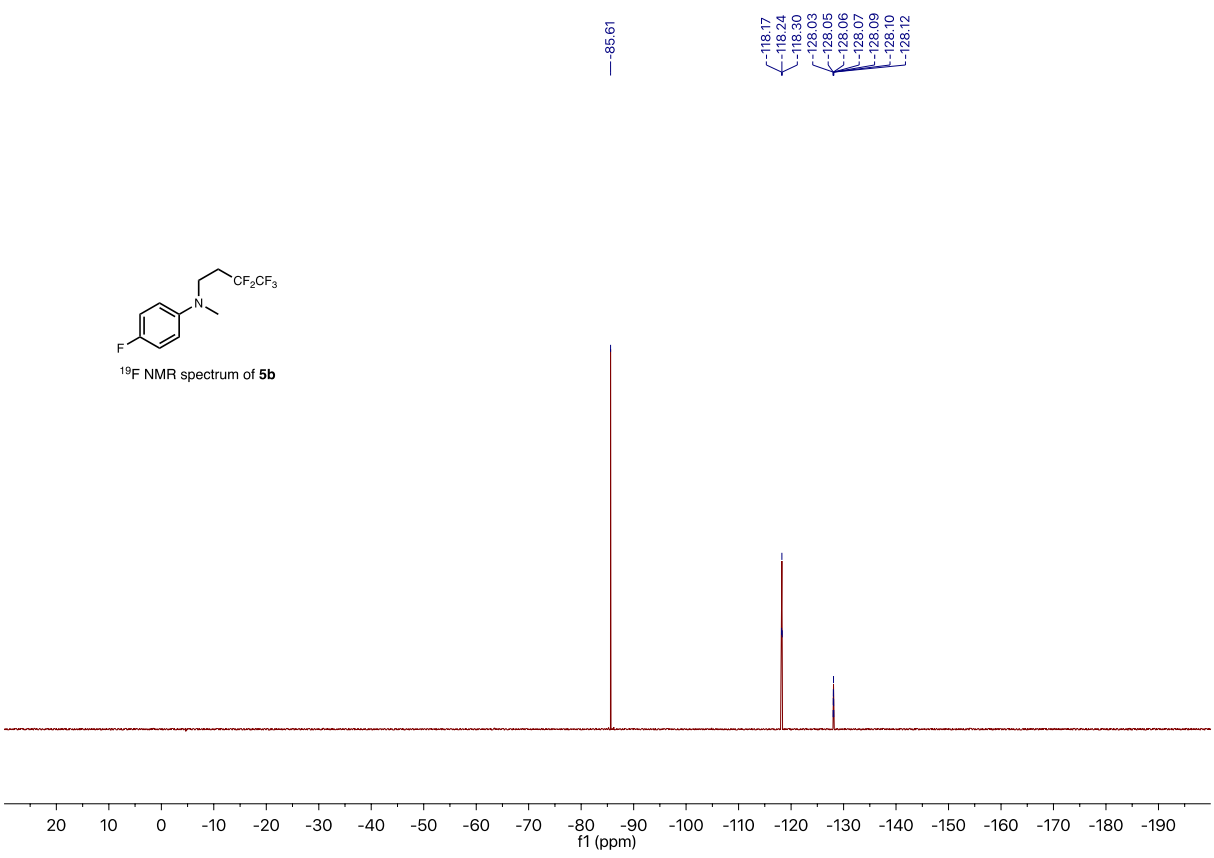
^{19}F NMR spectrum of **5a**



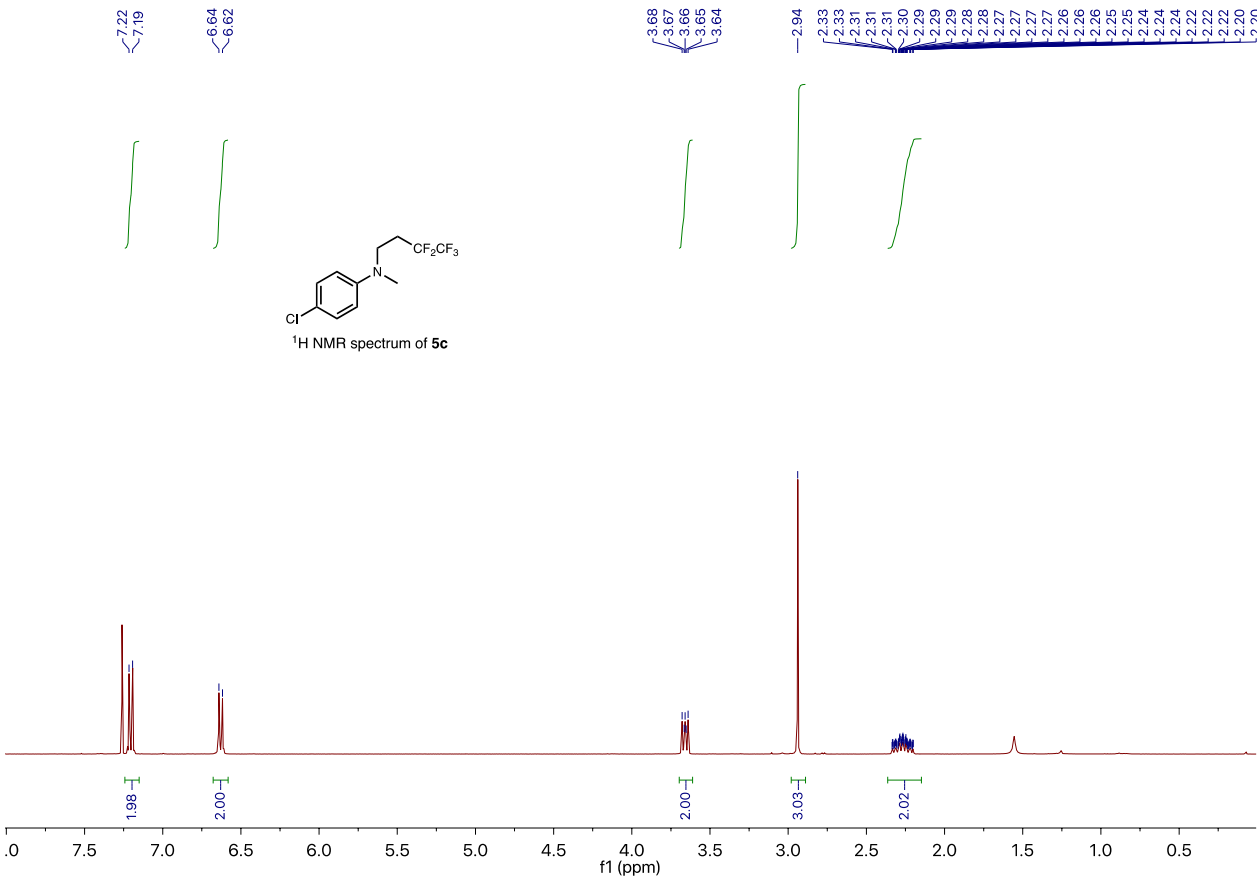
^{1}H NMR spectrum of **5b**



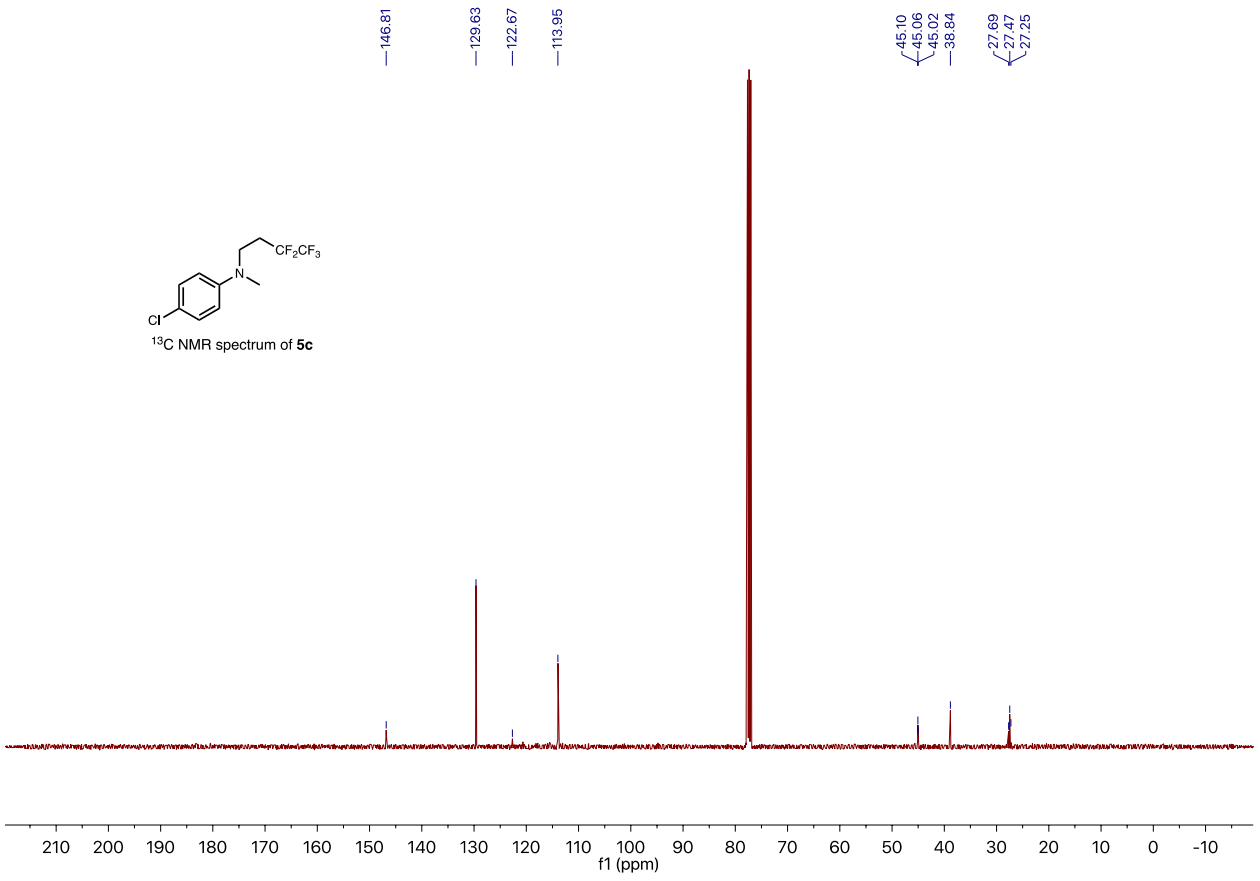
^{13}C NMR spectrum of **5b**



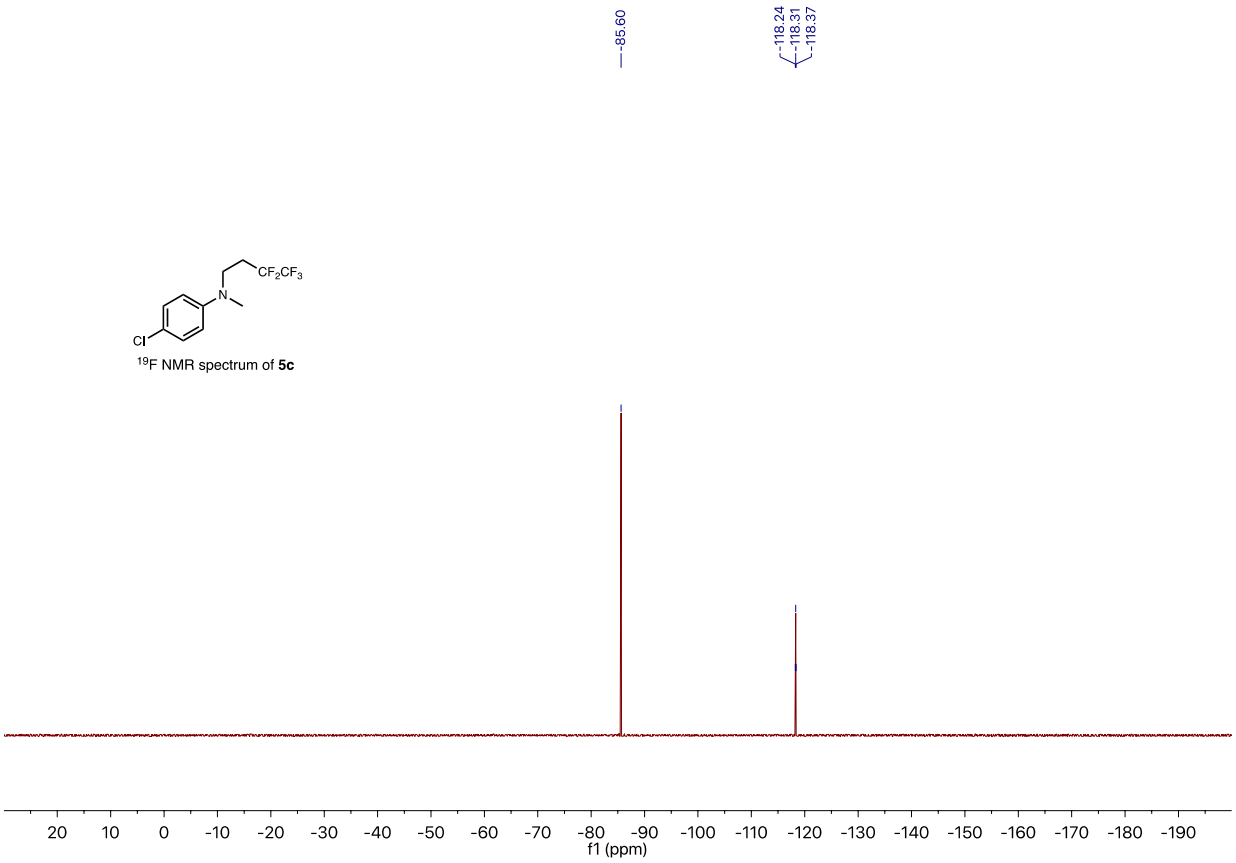
^{19}F NMR spectrum of **5b**



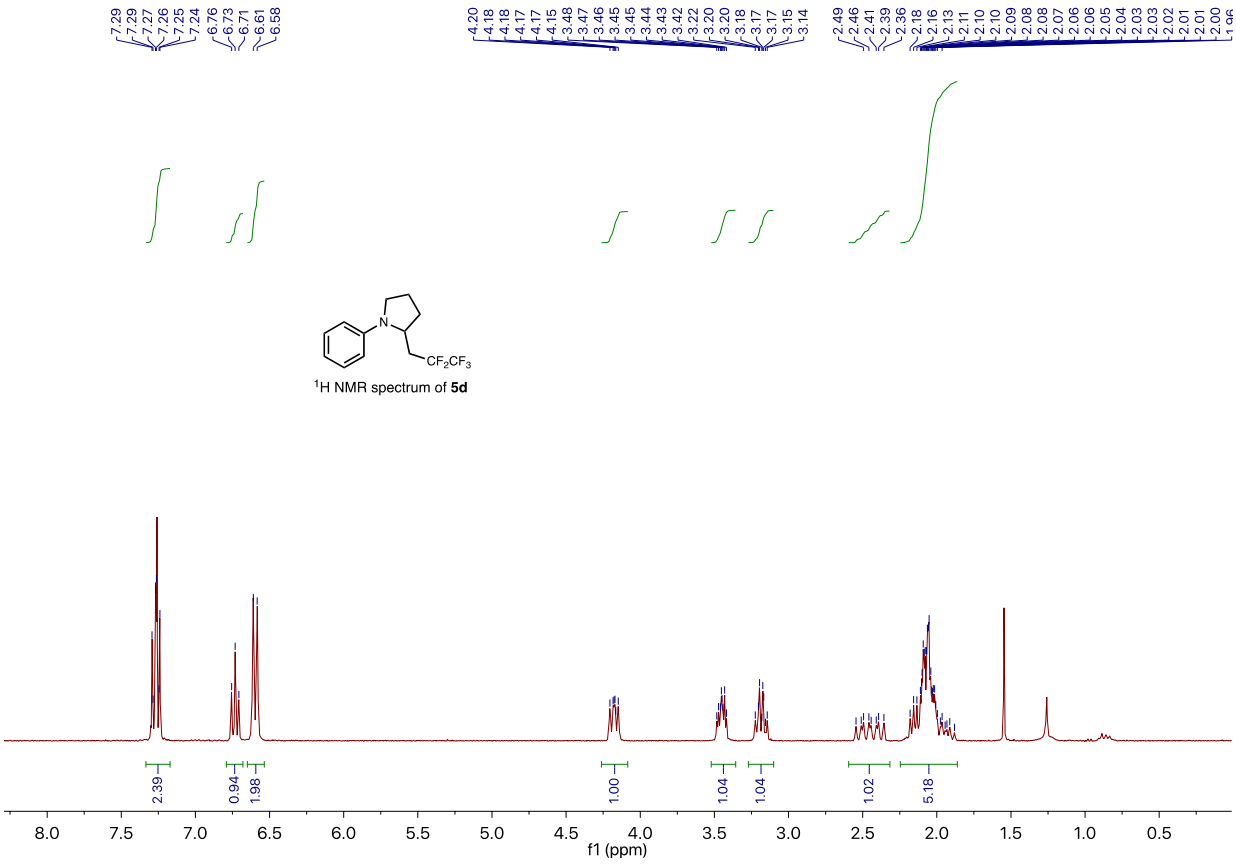
^{1}H NMR spectrum of **5c**



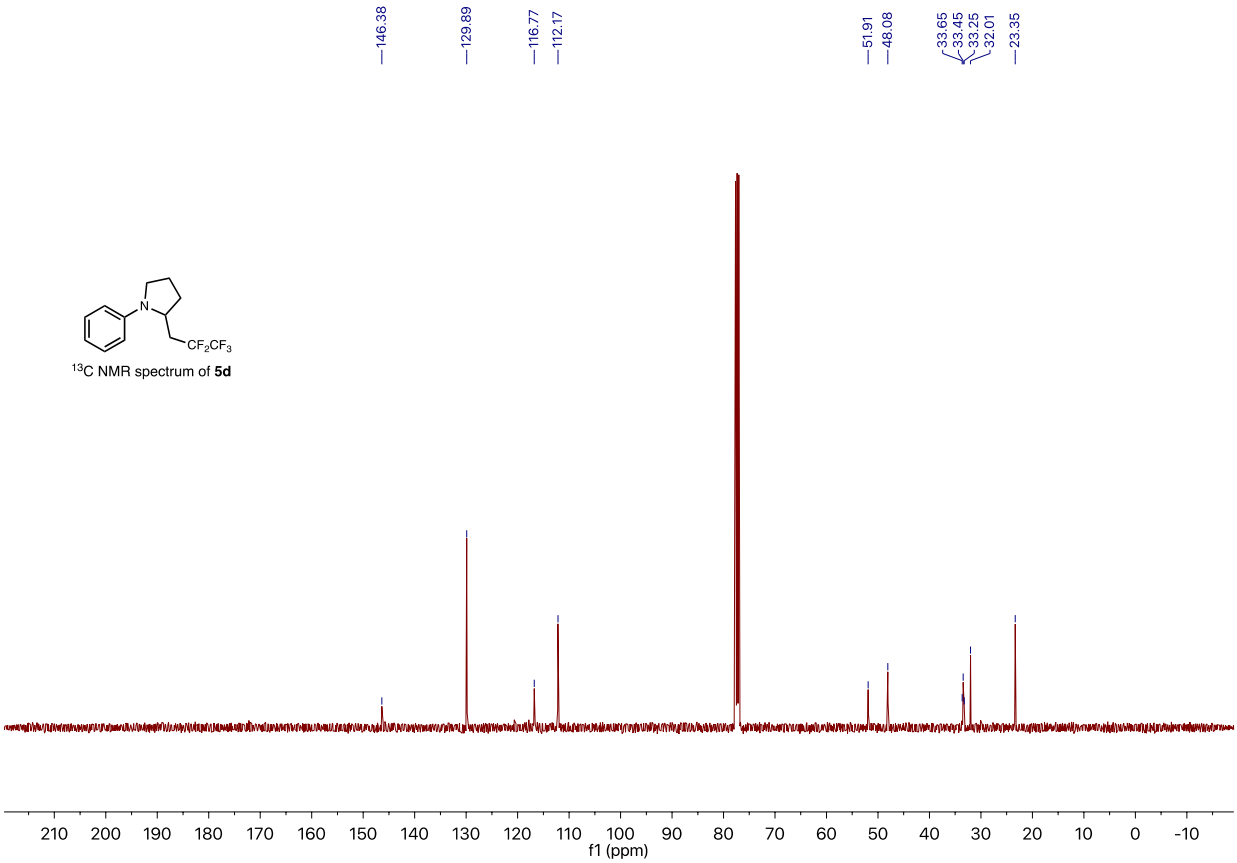
^{13}C NMR spectrum of **5c**



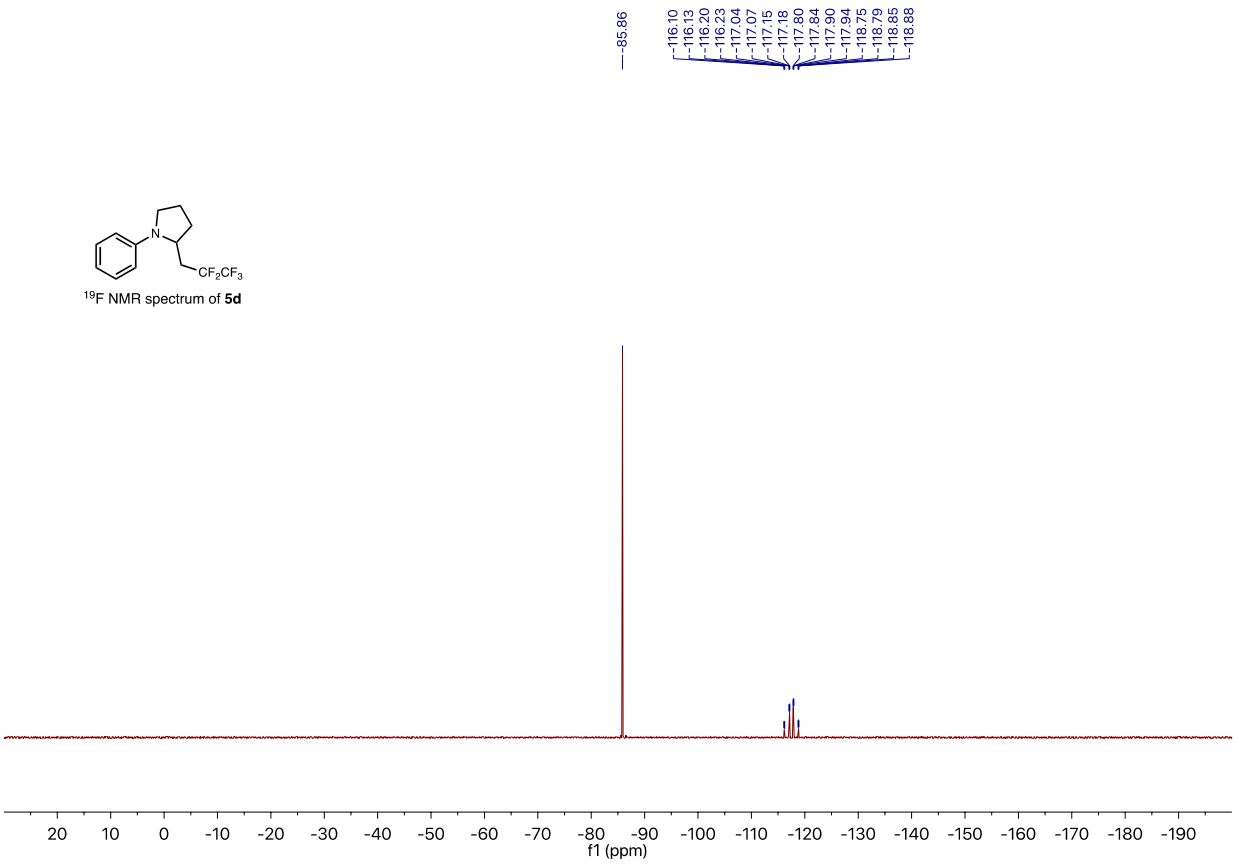
^{19}F NMR spectrum of **5c**



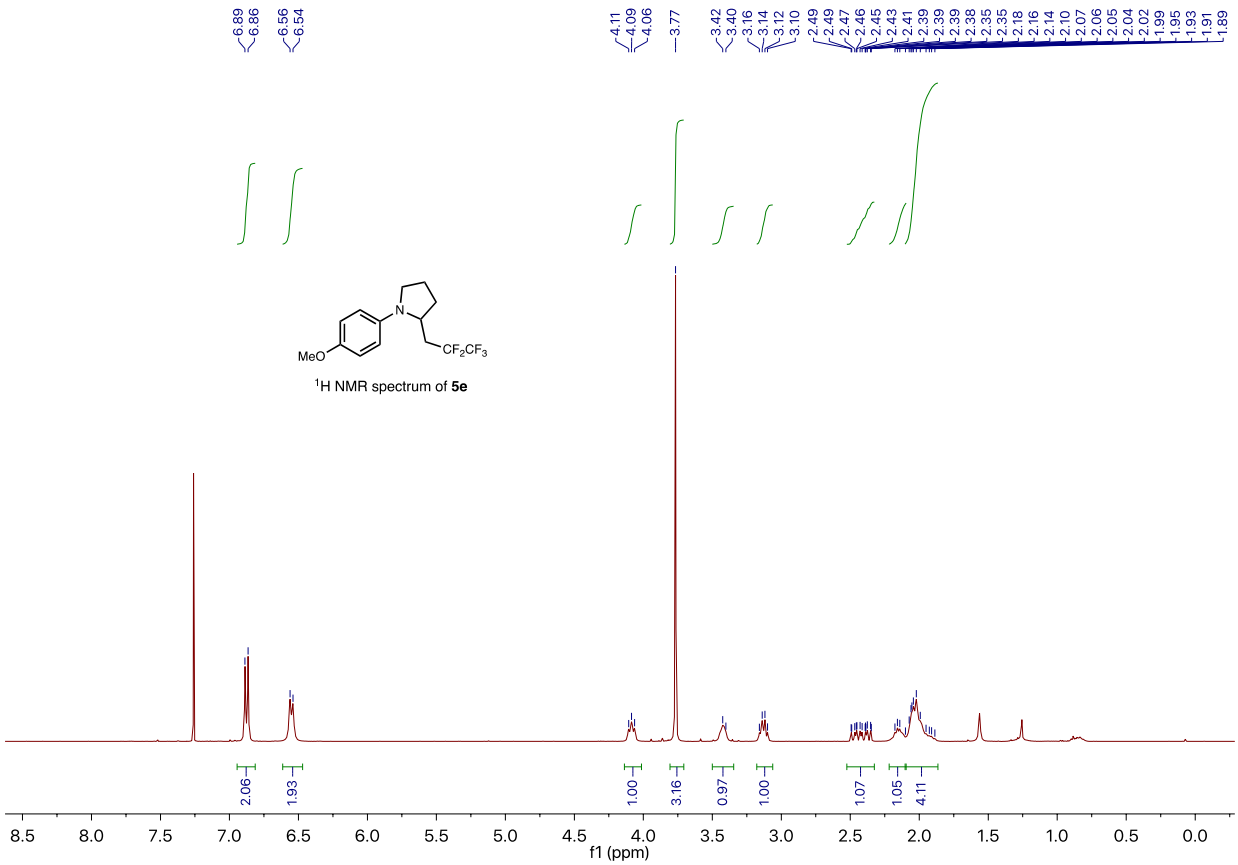
^{1}H NMR spectrum of **5d**



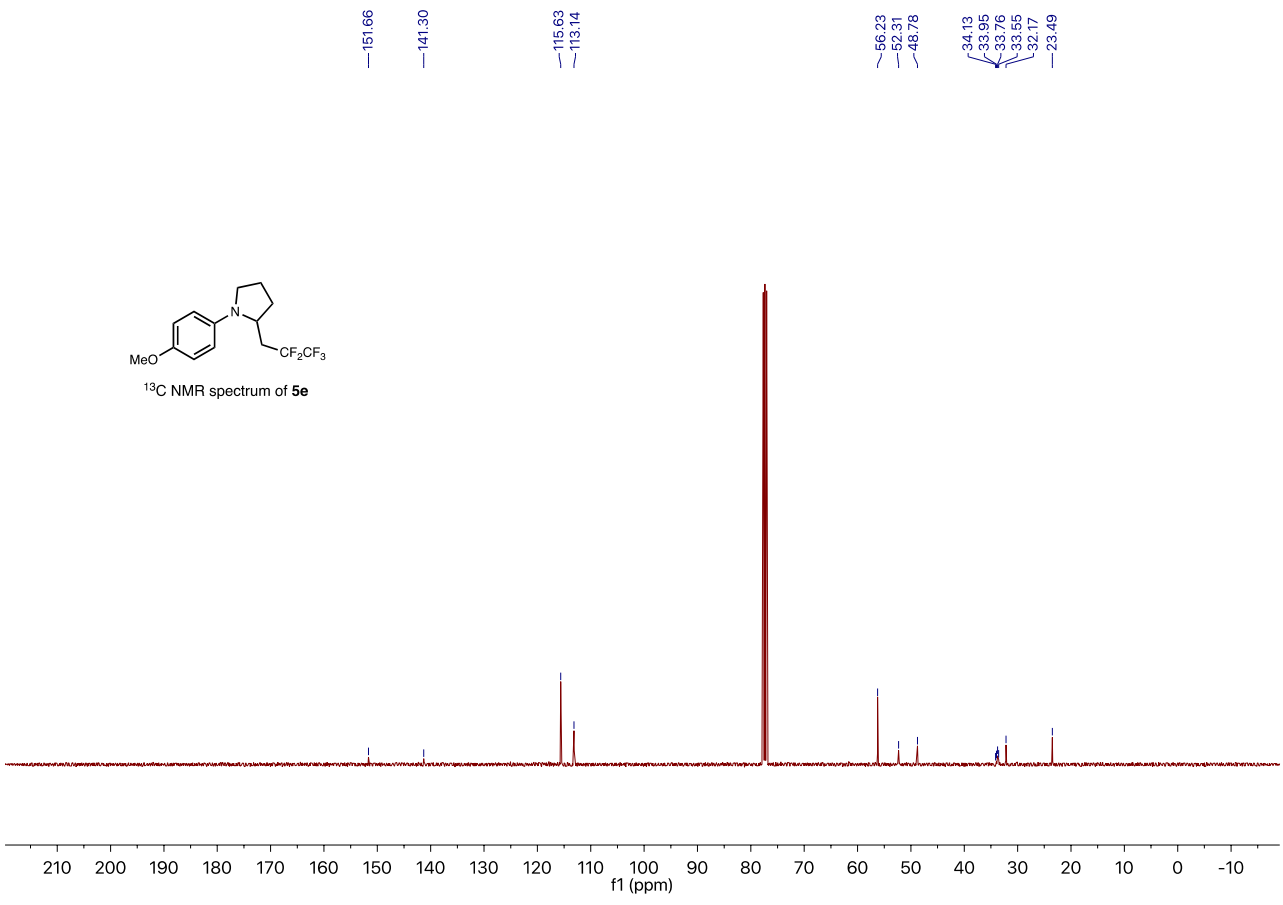
^{13}C NMR spectrum of **5d**



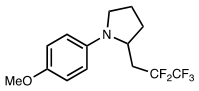
^{19}F NMR spectrum of **5d**



^{1}H NMR spectrum of **5e**



^{13}C NMR spectrum of **5e**



^{19}F NMR spectrum of **5e**